NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF *GINKGO BILOBA* EXTRACT

(CAS NO. 90045-36-6)

IN F344/N RATS AND B6C3F1/N MICE

(GAVAGE STUDIES)

Scheduled Peer Review Date: February 8-9, 2012

NOTICE

This DRAFT Technical Report is distributed solely for the purpose of predissemination peer review under the applicable information quality guidelines. It has not been formally disseminated by the NTP. It does not represent and should not be construed to represent NTP determination or policy.

NTP TR 578

NIH Publication No. 12-5920

National Toxicology Program

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

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

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CONTENTS

ABSTRACT

***GINKGO BILOBA* EXTRACT**

CAS No. 90045-36-6

Synonyms: Ginkgo, Ginkgo biloba, fossil tree, maidenhair tree, Japanese silver apricot, baiguo, bai guo ye, kew tree, yinhsing (yin-hsing)
Botanical name: *Ginkgo biloba*

Ginkgo biloba extract has been used primarily as a medicinal agent in the treatment or prevention of cardiovascular and cerebrovascular dysfunction. *Ginkgo biloba* extract was nominated for study by the National Institute of Environmental Health Sciences because of its widespread use as an herbal supplement to promote mental function and the limited availability of toxicity and carcinogenicity data. Furthermore, one of the major ingredients in *Ginkgo biloba* extract, quercetin, is a known mutagen. The *Ginkgo biloba* extract used in the current studies was procured from a supplier known to provide material to United States companies and contained 31.2% flavonol glycosides, 15.4% terpene lactones (6.94% bilobalide, 3.74% ginkgolide A, 1.62% ginkgolide B, 3.06% ginkgolide C), and 10.45 ppm ginkgolic acid. Male and female F344/N rats and B6C3F1/N mice were administered *Ginkgo biloba* extract in corn oil by gavage for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes.

3-MONTH STUDY IN RATS

Groups of 10 male and 10 female rats were administered 0, 62.5, 125, 250, 500, or 1,000 mg *Ginkgo biloba* extract/kg body weight in corn oil by gavage, 5 days per week for 14 weeks. Additional groups of 10 male and 10 female rats (clinical pathology study) were administered the same doses, 5 days per week for 23 days. All rats survived to the end of the study. Mean body weights of all dosed groups were similar to those of the vehicle control groups. Liver weights of all dosed groups of males and females were significantly greater than those of the vehicle control groups.

The incidences of hepatocyte hypertrophy in all dosed groups of males and in 500 and 1,000 mg/kg females were significantly greater than those in the vehicle control groups; there was a dose-related increase in severity of this lesion in males. Hepatocyte fatty change occurred in all dosed males. The incidences of thyroid gland follicular cell hypertrophy were significantly increased in 500 and 1,000 mg/kg males and in 1,000 mg/kg females. The incidences of pigmentation in the olfactory epithelium of the nose were significantly increased in 500 and 1,000 mg/kg males and in females administered 125 mg/kg or greater.

3-MONTH STUDY IN MICE

Groups of 10 male and 10 female mice were administered 0, 125, 250, 500, 1,000, or 2,000 mg *Ginkgo biloba* extract/kg body weight in corn oil by gavage, 5 days per week for 14 weeks. One female mouse in the 1,000 mg/kg group died of a dosing accident during week 11. Mean body weights of 2,000 mg/kg females were significantly less than those of the vehicle control group. Ruffled fur was observed in two 1,000 mg/kg males between weeks 7 and 8 and all 2,000 mg/kg males between weeks 5 and 9. Liver weights of 250 mg/kg or greater males and all dosed groups of females were significantly greater than those of the vehicle control groups. Kidney weights of 2,000 mg/kg males were significantly less than those of the vehicle control group. The Markov transition matrix analyses indicate female mice in the 2,000 mg/kg group had a significantly higher probability of extended estrus than did the vehicle control females.

The incidences of hepatocytic hypertrophy were significantly increased in males and females in the 250 mg/kg or greater groups. Significantly increased incidences of focal hepatocytic necrosis occurred in 1,000 and 2,000 mg/kg males. The incidences of hyaline droplet accumulation in the respiratory epithelium of the nose were significantly increased in 500 mg/kg males and 1,000 and 2,000 mg/kg females. In the olfactory epithelium of the nose, the incidences of hyaline droplet accumulation were significantly increased in the 125 (female only), 500, and 1,000 mg/kg groups. Incidences of atrophy of the olfactory epithelium were significantly increased in the 1,000 mg/kg groups. The incidences of pigment accumulation in macrophages in the olfactory epithelium were significantly increased in males in the 500 mg/kg or greater groups and in 1,000 and 2,000 mg/kg females.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats were administered 0, 100, 300, or 1,000 mg *Ginkgo biloba* extract/kg body weight in corn oil by gavage, 5 days per week for 104 or 105 (females) weeks. Additional groups of 10 male and 10 female rats (special study) were administered the same doses, 5 days per week for 14 weeks. Survival of 1,000 mg/kg males was significantly less than that of the vehicle controls. At week 14, all dosed groups of males and 1,000 mg/kg females had increased levels of thyroid stimulating hormone compared to those of the vehicle control groups. There were no significant increases in the levels of triiodothyronine or total thyroxine. Mean body weights of 300 mg/kg males and females were less (10% or more) than those of the vehicle controls after week 93, and those of 1,000 mg/kg males and females were less after week 89. Clinical findings included ruffled fur in seven, eight, and 10 males in the 100, 300, and 1,000 mg/kg groups, respectively, beginning at week 89; four vehicle control males also had ruffled fur.

Liver weights were significantly increased in all dosed groups of special study rats at 14 weeks. In the liver at 2 years, incidences of hepatocellular adenoma were slightly increased in 100 and 300 mg/kg males. Significantly increased incidences of nonneoplastic lesions at 2 years included hepatocyte hypertrophy and bile duct hyperplasia in all dosed groups of males and females, focal fatty change in all dosed groups of females, cystic degeneration in 100 and 1,000 mg/kg males, and oval cell hyperplasia and necrosis in 1,000 mg/kg males.

In the thyroid gland, incidences of follicular cell adenoma were slightly increased in 300 and 1,000 mg/kg males and 300 mg/kg females. Single incidences of follicular cell carcinoma occurred in the 300 and 1,000 mg/kg female groups. There were significantly increased incidences of follicular cell hypertrophy in all dosed groups of males and females and follicle hyperplasia in all dosed groups of males.

In the nose, two incidences of adenoma of the respiratory epithelium occurred in 300 mg/kg females. Except for respiratory epithelium hyperplasia in 100 mg/kg females, the incidences of transitional epithelium and respiratory epithelium hyperplasia were significantly increased in all dosed groups of males and females. Except for olfactory epithelium respiratory metaplasia in 100 mg/kg females, the incidences of atrophy, respiratory metaplasia, nerve atrophy, and pigmentation were significantly increased in the olfactory epithelium of all dosed groups of males and females. Incidences of goblet cell hyperplasia in the respiratory epithelium were significantly increased in 300 and 1,000 mg/kg males and females, and incidences of chronic active inflammation were significantly increased in 1,000 mg/kg males and females. The incidence of submucosa fibrosis was significantly increased in 1,000 mg/kg males.

The incidences of mononuclear cell leukemia in 300 and 1,000 mg/kg males were significantly greater than that in the vehicle controls.

Dose-related increased severity of kidney nephropathy was noted in all dosed groups of males.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice were administered 0, 200, 600, or 2,000 mg *Ginkgo biloba* extract/kg body weight in corn oil by gavage, 5 days per week for 104 weeks. Survival of 600 and 2,000 mg/kg males was significantly less than that of the vehicle controls; survival of 600 mg/kg females was significantly greater than that of the vehicle controls. Mean body weights of 600 and 2,000 mg/kg males were less (10% or more) than those of the vehicle controls after weeks 85 and 77, respectively; mean body weights of 2,000 mg/kg females were generally less than those of the vehicle controls between weeks 17 and 69 and after week 93.

In the liver, there were significantly increased incidences of hepatocellular adenoma in all dosed groups of females, hepatocellular carcinoma in all dosed groups of males and 2,000 mg/kg females, and hepatoblastoma in all dosed groups of males and 600 and 2,000 mg/kg females. The increased incidences of these neoplasms were primarily due to increased incidences of multiple adenoma, carcinoma, and hepatoblastoma. Except for the incidences of hepatocellular carcinoma or hepatoblastoma (combined) in 200 and 600 mg/kg females, the incidences of hepatocellular adenoma or carcinoma (combined), hepatocellular carcinoma or hepatoblastoma (combined), and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) were significantly increased in all dosed groups of males and females. Significantly increased incidences of nonneoplastic liver lesions included hypertrophy in all dosed groups of males and females, erythrophagocytosis in all dosed groups of males and in 600 and 2,000 mg/kg females, hematopoietic cell proliferation, inflammation, and necrosis in 600 and 2,000 mg/kg males, and cytoplasmic vacuolization, eosinophilic focus, and mixed cell focus in all dosed groups of females.

In the thyroid gland, two incidences each of follicular cell adenoma occurred in the 600 and 2,000 mg/kg male groups. The incidence of follicle hyperplasia was significantly increased in 2,000 mg/kg males, and the incidences of follicular cell hypertrophy were significantly increased in 2,000 mg/kg males and 600 and 2,000 mg/kg females.

In the forestomach, the incidences of inflammation, epithelium hyperplasia, and epithelium hyperkeratosis were significantly increased in all dosed groups of males and in 2,000 mg/kg females; the incidences of epithelium ulcer were significantly increased in 2,000 mg/kg males and females.

In the nose, the incidences of hyaline droplet accumulation in the olfactory epithelium were significantly increased in 2,000 mg/kg males and females; the incidences of pigmentation in the olfactory epithelium were significantly increased in 2,000 mg/kg males and 600 and 2,000 mg/kg females.

GENETIC TOXICOLOGY

Ginkgo biloba extract was mutagenic in *S. typhimurium* strains TA98 and TA100, and in *E. coli* strain WP2 *uvrA*/pKM101, with and without exogenous metabolic activation. Results of a peripheral blood micronucleus

test in male and female B6C3F1/N mice administered *Ginkgo biloba* extract for 3 months by gavage were negative in males but judged to be equivocal in females based on a significant trend test.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity** of *Ginkgo biloba* extract in male F344/N rats based on increased incidences of thyroid gland follicular cell adenoma. The increased incidences of mononuclear cell leukemia and hepatocellular adenoma may have been related to *Ginkgo biloba* extract administration. There was *some evidence of carcinogenic activity* of *Ginkgo biloba* extract in female F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. Increased occurrence of respiratory epithelium adenomas in the nose may have been related to *Ginkgo biloba* extract administration. There was *clear evidence of carcinogenic activity* of *Ginkgo biloba* extract in male B6C3F1/N mice based on increased incidences of hepatocellular carcinoma and hepatoblastoma. The increased incidences of thyroid gland follicular cell adenoma were also related to *Ginkgo biloba* extract administration. There was *clear evidence of carcinogenic activity* of *Ginkgo biloba* extract in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma.

Administration of *Ginkgo biloba* extract resulted in increased incidences of nonneoplastic lesions in the liver, thyroid gland, and nose of male and female rats and mice and the forestomach of male and female mice. Increased severity of nephropathy in male rats was also due to administration of *Ginkgo biloba* extract.
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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 15.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of *Ginkgo biloba* Extract

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Doses in corn oil by gavage	0, 100, 300, or 1,000 mg/kg	0, 100, 300, or 1,000 mg/kg	0, 200, 600, or 2,000 mg/kg	0, 200, 600, or 2,000 mg/kg
Body weights	300 mg/kg group ≥10% less than the vehicle control group after week 93; 1,000 mg/kg group ≥10% less than the vehicle control group after week 89	300 mg/kg group ≥10% less than the vehicle control group after week 93; 1,000 mg/kg group ≥10% less than the vehicle control group after week 89	600 and 2,000 mg/kg groups ≥10% less than the vehicle control groups after week 85 and 77, respectively	2,000 mg/kg group ≥10% less than the vehicle control group between weeks 17 and 69 and after week 93
Survival rates	38/50, 37/50, 31/50, 16/50	37/50, 27/50, 37/50, 32/50	34/50, 27/50, 21/50, 23/50	31/50, 36/50, 43/50, 36/50
Nonneoplastic effects	Liver: hepatocyte, hypertrophy (1/50, 17/50, 26/50, 27/50); bile duct, hyperplasia (32/50, 43/50, 46/50, 46/50); oval cell, hyperplasia (0/50, 1/50, 1/50, 10/50); degeneration, cystic (4/50, 14/50, 10/50, 14/50); necrosis (1/50, 4/50, 6/50, 7/50) Thyroid gland: follicular cell hypertrophy (13/50, 37/50, 41/49, 41/45); follicle, hyperplasia (0/50, 7/50, 9/49, 5/45) Kidney: severity of nephropathy (1.7, 2.0, 2.4, 2.9) Nose: transitional epithelium, hyperplasia (2/50, 18/49, 43/49, 31/50); respiratory epithelium, hyperplasia (14/50, 28/49, 45/49, 35/50); olfactory epithelium, atrophy (1/50, 26/49, 37/49, 31/50); olfactory epithelium, respiratory metaplasia (9/50, 30/49, 40/49, 32/50); nerve, olfactory epithelium, atrophy (0/50, 17/49, 14/49, 23/50); olfactory epithelium, pigmentation (0/50, 39/49, 42/49, 30/50); inflammation, chronic active (33/50, 32/49, 38/49, 46/50); goblet cell, respiratory epithelium, hyperplasia (20/50, 18/49, 41/49, 34/50); submucosa, fibrosis (0/50, 0/49, 0/49, 8/50)	Liver: hepatocyte, hypertrophy (7/50, 15/50, 27/50, 33/50); bile duct, hyperplasia (11/50, 31/50, 31/50, 33/50); fatty change, focal (11/50, 25/50, 30/50, 25/50) Thyroid gland: follicular cell, hypertrophy (15/49, 41/50, 45/49, 48/49) Nose: transitional epithelium, hyperplasia (0/49, 6/49, 32/50, 36/46); respiratory epithelium, hyperplasia (9/49, 6/49, 19/50, 34/46); olfactory epithelium, atrophy (0/49, 18/49, 25/50, 37/46); olfactory epithelium, respiratory metaplasia (8/49, 4/49, 32/50, 37/46); nerve, olfactory epithelium, atrophy (0/49, 15/49, 22/50, 33/46); olfactory epithelium, pigmentation (0/49, 37/49, 43/50, 40/46); inflammation, chronic active (22/49, 16/49, 26/50, 38/46); goblet cell, respiratory epithelium, hyperplasia (6/49, 2/49, 18/50, 35/46)	Liver: hypertrophy (3/50, 19/50, 35/50, 23/50); erythrophagocytosis (0/50, 4/50, 11/50, 7/50); hematopoietic cell proliferation (4/50, (9/50, 12/50, 14/50); inflammation (28/50, 35/50, 42/50, 39/50); necrosis (9/50, 15/50, 17/50, 19/50) Thyroid gland: follicle hyperplasia (2/49, 1/49, 7/50, 25/50); follicular cell hypertrophy (2/49, 0/49, 2/50, 38/50) Forestomach: inflammation (11/50, 24/50, 21/50, 45/50); epithelium hyperplasia (14/50, 27/50, 27/50, 45/50); epithelium hyperkeratosis (11/50, 24/50, 24/50, 46/50); epithelium ulcer (7/50, 10/50, 12/50, 24/50) Nose: olfactory epithelium, hyaline droplet accumulation (18/50, 16/50, 15/50, 28/50); olfactory epithelium, pigmentation (0/50, 1/50, 3/50, 13/50)	Liver: hypertrophy (0/50, 18/50, 37/50, 37/50); erythrophagocytosis (0/50, 3/50, 7/50, 16/50); vacuolization cytoplasmic (18/50, 38/50, 44/50, 35/50); eosinophilic focus (26/50, 39/50, 43/50, 45/50); mixed cell focus (7/50, 27/50, 31/50, 31/50) Thyroid gland: follicular cell hypertrophy (1/49, 5/48, 9/49, 39/48) Forestomach: inflammation (4/50, 6/50, 5/50, 19/50); epithelium hyperplasia (8/50, 18/50, 11/50, 20/50); epithelium hyperkeratosis (3/50, 11/50, 5/50, 20/50); epithelium ulcer (1/50, 1/50, 1/50, 11/50) Nose: olfactory epithelium, hyaline droplet accumulation (5/50, 3/50, 12/50, 17/50); olfactory epithelium, pigmentation (0/50, 1/50, 6/50, 13/50)
Neoplastic effects	Thyroid gland: follicular cell, adenoma (2/50, 1/50, 3/49, 5/45)	Thyroid gland: follicular cell, adenoma (1/49, 0/50, 3/49, 1/49); follicular cell, carcinoma (0/49, 0/50, 1/49, 1/49)	Liver: hepatocellular carcinoma (22/50, 31/50, 41/50, 47/50); hepatoblastoma (3/50, 28/50, 36/50, 38/50) Thyroid gland: follicular cell, adenoma (0/50, 0/50, 2/50, 2/50)	Liver: hepatocellular adenoma (17/50, 37/50, 41/50, 48/50); hepatocellular carcinoma (9/50, 10/50, 15/50, 44/50); hepatoblastoma (1/50, 1/50, 8/50, 11/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of *Ginkgo biloba* Extract

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Equivocal findings	Mononuclear cell leukemia: (9/50, 12/50, 22/50, 21/50) Liver: hepatocellular adenoma (0/50, 3/50, 3/50, 0/50)	Nose: respiratory epithelium, adenoma (0/49, 0/49, 2/50, 0/46)	None	None
Level of evidence of carcinogenic activity	Some evidence	Some evidence	Clear evidence	Clear evidence
Genetic toxicology				
Bacterial gene mutations:	Positive in *S. typhimurium* strains TA98 and TA100 with and without S9; positive in *E. coli* strain WP2 *uvr*A/pKM101 with and without S9			
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:	Negative in males and equivocal in females			

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 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 on 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.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

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 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 TECHNICAL REPORTS
PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Technical Report on *Ginkgo biloba* extract on February 8-9, 2012, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities 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.

NOTE: The list of Peer Review Panel members will appear in a future draft of this report.

SUMMARY OF PEER REVIEW PANEL COMMENTS

NOTE: A summary of the Peer Review Panel's remarks will appear in a future draft of this report.

INTRODUCTION

***GINKGO BILOBA* EXTRACT**

CAS No. 90045-36-6

Synonyms: Ginkgo, Ginkgo biloba, fossil tree, maidenhair tree, Japanese silver apricot, baiguo, bai guo ye, kew tree, yinhsing (yin-hsing)
Botanical name: *Ginkgo biloba*

CHEMICAL AND PHYSICAL PROPERTIES

The ginkgo tree is often referred to as a "living fossil" because *Ginkgo* species have been identified from as early as the Jurassic Period 170 million years ago (Zhou and Zheng, 2003). The sole surviving member of the Ginkgoaceae family, *Ginkgo biloba*, is a highly adaptable and hardy tree that is dioecious, having separate male and female plants. Some specimens are over 1,000 years old (Del Tredici, 1991; Mahadevan and Park, 2008). Ginkgo trees begin reproducing after approximately 20 years of maturation, at which time the female produces fruit that is distinctly malodorous (Mahadevan and Park, 2008). The unique, bilobed leaves of *Ginkgo biloba* are fan-shaped and are similar in appearance to those of the maidenhair fern, giving rise to the common name "maidenhair tree" (Mahadevan and Park, 2008).

Like other herbals, *Ginkgo biloba* leaves contain a complex mixture of chemical constituents, which can vary depending on the strain of ginkgo, conditions of growth, time of harvest, etc. (Smith and Luo, 2004). The main chemical constituents in *Ginkgo biloba* leaves include terpene trilactones, flavonol glycosides, biflavones, proanthocyanidins, alkylphenols, phenolic acids, and polyprenols (van Beek and Montoro, 2009). The main constituents of *Ginkgo biloba* leaves and their concentrations in standardized *Ginkgo biloba* extract (EGb 761®) and other commercially available preparations are shown in Table 1. Most of the compounds that have been identified in ginkgo are commonly found in the leaves of other higher plants. However, the terpene trilactones represent a group of chemicals that are unique to the ginkgo (Hasler, 2000).

The terpene trilactones and flavonol glycosides are generally regarded as the major contributors to the positive biological effects attributed to ginkgo (van Beek and Montoro, 2009), whereas, ginkgolic acids are typically regarded as causing negative biological activities (e.g., cytotoxicity, mutagenicity) (Westendorf and Regan, 2000).

TABLE 1
Constituents of *Ginkgo biloba* Extract[a]

Class	Identified Chemical Constituents	Target Specification in EGb 761® (range in other preparations)
Terpene trilactones	Ginkgolides A, B, C, J, K, L, M (found in root only); bilobalide (sesquiterpene)	6% (0.2%-11%)
Flavonol glycosides	Major flavonoids: quercetin, kaempferol, isorhamnetin Minor flavonoids: apigenin, luteolin, myricetin[b]	24% (24%-36%)
Biflavones	Bilobetin, ginkgetin, isoginkgetin, sciadopitysin	0% (0.05%-1.7%)
Proanthocyanidins	Dimers of procyanidin and prodelphinidin classes	7% (4%-12%)
Alkylphenols	Ginkgolic acids, cardanols	≤ 5 ppm (0.5%-4.8% in leaves; <500 to approximately 90,000 ppm)
Carboxylic acids	Non-phenolic acids (ascorbic acid, D-glucaric acid, quinic acid, shikimic acid), phenolic acids (protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic and chlorogenic acids)	13% (N/A)
Flavanols	Catechin, epicatechin, gallocatechin, and epigallocatechin[b]	2% (N/A)
Polyprenols	C_{85}, C_{90}, C_{95} polyprenol[c]	0% (1.9%-2.0% in leaves)

[a] Kressmann *et al*., 2002; van Beek, 2002; van Beek and Montoro, 2009; Gawron-Gzella *et al*., 2010
[b] Hasler *et al*., 1992
[c] Huh *et al*., 1992

Ginkgotoxin, or 4′-*O*-methylpyridoxine, is also associated with toxicity, but is found primarily in ginkgo seeds and only in lesser amounts in the leaves and extracts prepared from the leaves (Arenz *et al*., 1996). In recent years, the use of fingerprinting to quantify key chemical constituents of plant-based formulations has been advocated as a quality control measure to ensure appropriate quantities of the biologically-active constituents and a lack of ginkgolic acids (van Beek and Montoro, 2009).

PRODUCTION, USE, AND HUMAN EXPOSURE

The attractive appearance of ginkgo trees and their robust nature have lead to widespread cultivation of *Ginkgo biloba* as an ornamental plant, expanding its range from its origins in China to every country in the temperate zone (Del Tredici, 1991). In addition to its horticultural popularity, various parts of the *Ginkgo biloba* plant have been used for food or medicine. Ginkgo seeds are regularly consumed in Japan, Korea, and China, despite the fact that poisoning, manifesting as gastrointestinal distress, irritability, and tonic or clonic seizures, can result from overconsumption of seeds due to their high 4′-*O*-methylpyridoxine content (Kajiyama *et al*., 2002; Kobayashi *et al*., 2011). Seeds from the ginkgo tree have been used medicinally dating back thousands of years. In Chinese medicine, *Ginkgo biloba* seeds have been used to treat pulmonary issues, alcohol abuse, and bladder infections, while ginkgo leaves came into use later to treat skin infections, as well as heart and lung disease (Mahady, 2002; Smith and Luo, 2004). Current use of *Ginkgo biloba* extract centers on leaf-based preparations for the promotion of circulation and brain function, and the treatment of tinnitus.

In 1965, the German physician-pharmacist Dr. Willmar Schwabe III developed *Ginkgo biloba* leaf extracts (De Feudis, 2003). The final product, a standardized *Ginkgo biloba* extract (EGb 761®), has been subsequently marketed by Dr. Willmar Schwabe Pharmaceuticals under the trade names Ginkgold® (Nature's Way™), Kaveri®, Rökan®, Tanakan®, and Tebonin®. EGb 761® is standardized to contain 24% flavone glycosides (quercetin, kaempferol, isorhamnetin), 6% terpene lactones [3.1% ginkgolides (A, B, C, J) and 2.9% bilobalide], various organic acids (5% to 10%), and other constituents (Table 1). Many *Ginkgo biloba* components are biologically active, and it is believed that the action of multiple constituents contributes to the medicinal properties of the plant. However, the standardization of EGb 761® and other *Ginkgo biloba* extracts is based on their flavonoid and terpene

trilactone contents (Figure 1), as these compounds are thought to be primarily responsible for the pharmacological activity associated with *Ginkgo biloba* extract.

In the United States, herbal formulations sold as dietary supplements such as *Ginkgo biloba* extract are regulated under the Dietary Supplement Health and Education Act of 1994 (DSHEA). As such, they are not subject to the same standards of pre-market testing as drugs intended to treat, cure, prevent, diagnose, or mitigate disease. In contrast, in Germany and France *Ginkgo biloba* extract is regulated as a prescription drug and therefore, requires registration and adherence to specified content standards. For *Ginkgo biloba* extracts, these are 22% to 27% flavone glycosides, 5% to 7% terpene lactones (2.8% to 3.4% ginkgolides A, B, C and 2.6% to 3.2% bilobalide), and not more than 5 ppm ginkgolic acids, due to their cytotoxic and allergenic potential (Kressmann *et al*., 2002). In the United States, a wide range of component concentrations is observed in available *Ginkgo biloba* products (Table 1) (Kressmann *et al*., 2002). However, analyses by independent investigators showed variation even in the composition of the standardized extracts (Woerdenbag and van Beek, 1997).

In surveys addressing complementary and alternative medicine use in the United States, *Ginkgo biloba* is among the top five most used herbal supplements on the market (Kennedy, 2005; Abebe *et al*., 2011). Kennedy (2005) estimated that of the 38 million Americans that used herbal medicines in 2002, 20.1% of that population, or approximately 7.7 million people, took *Ginkgo biloba* extracts. Although sales data are difficult to obtain, one source cites a figure of over $249 million per year in United States sales of *Ginkgo biloba* extracts (DeKosky *et al*., 2008).

The therapeutic effect most frequently ascribed to ginkgo use in popular culture is improvement of memory and brain function, however, there are numerous other health benefits attributed to its use. In Germany, it is indicated for the treatment of intermittent claudication, decreased mental function (including forgetfulness, early dementia, and concentration problems), and tinnitus (van Beek and Montoro, 2009). *Ginkgo biloba* extract has been studied in humans as a treatment for the neurological effects associated with Alzheimer's disease, traumatic brain injury, stroke, dementia, normal aging, edema, tinnitus, and macular degeneration (Diamond *et al*., 2000; McKenna *et al*.,

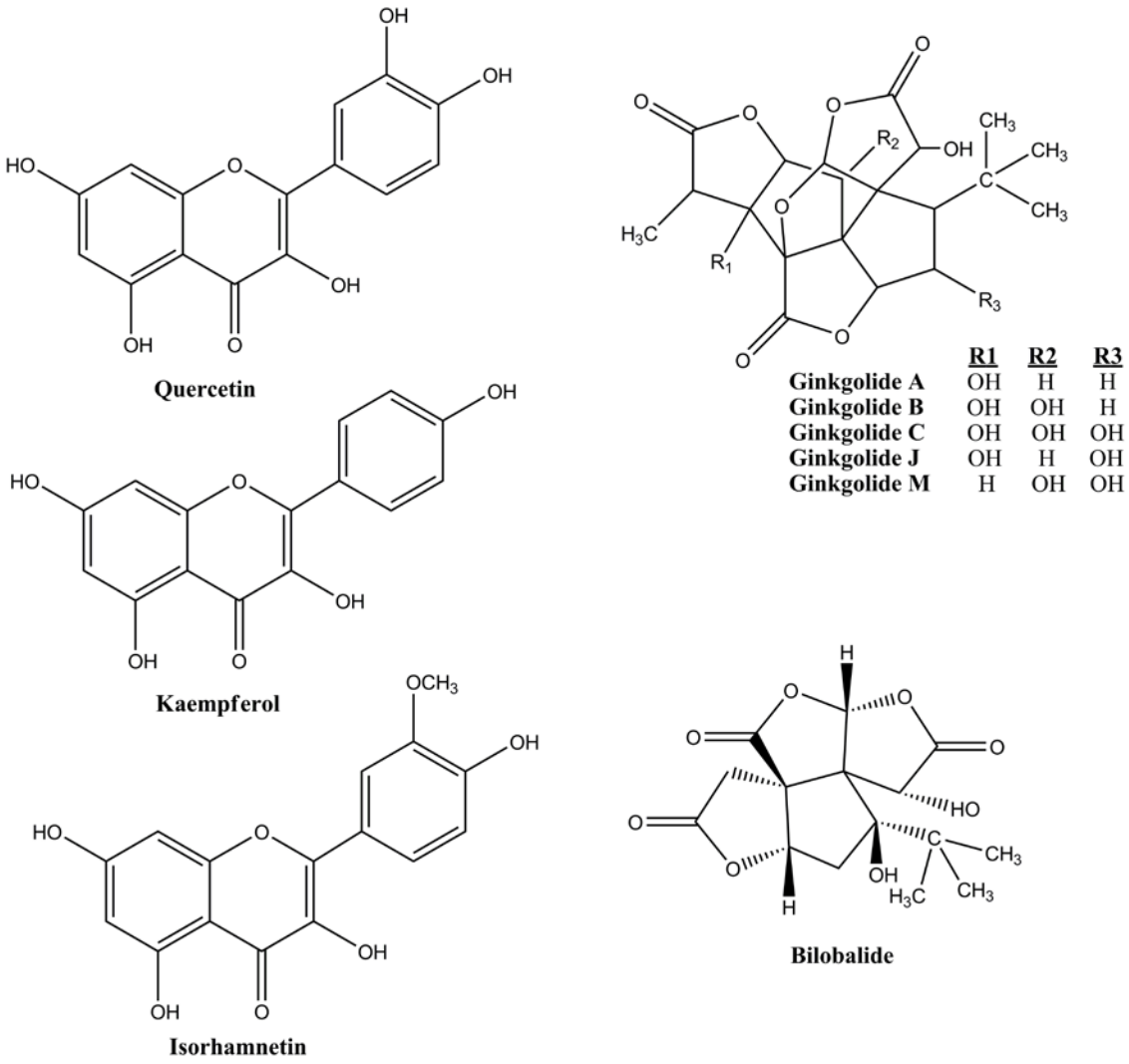


FIGURE 1
Structures of Flavonoid and Terpene Trilactone Contents of Standardized *Ginkgo biloba* Extract

2001). Additionally, preclinical *in vitro* or experimental animal studies have explored the use of *Ginkgo* for cardiovascular indications (thrombosis, embolism), anxiety/stress, sexual dysfunction, and cancer (McKenna *et al*., 2001). Despite the popular use of *Ginkgo biloba* extract for numerous ailments, the largest clinical trial of ginkgo effects (Ginkgo Evaluation of Memory Study) failed to find an effect of *Ginkgo biloba* on prevention of dementia

(DeKosky *et al*., 2008), prevention of cognitive decline (Snitz *et al*., 2009), reduction in cardiovascular disease-related events or mortality (Kuller *et al*., 2010), or decreases in blood pressure or hypertension (Brinkley *et al.*, 2010).

The diverse conditions for which *Ginkgo biloba* has been explored as a potential treatment are paralleled by a complex array of mechanisms of action associated with various *Ginkgo biloba* components. Although individual components have been found to be pharmacologically active, the multifaceted action of *Ginkgo biloba* is likely due to multiple components and interactions that have yet to be fully elucidated (De Feudis and Drieu, 2000).

The two mechanisms of action most often associated with the proclaimed health benefits of *Ginkgo biloba* extract are the antagonism of Platelet Activating Factor (PAF) by the ginkgolides and the antioxidant action of the flavonoids (Smith and Luo, 2004). However, there are multiple mechanisms of action that are likely to impact disease pathways. Several mechanisms of action identified through *in vitro* or *in vivo* studies are hypothesized to play a role in the health promoting action of *Ginkgo biloba* extract. These include: antagonism of PAF by the ginkgolides (Smith *et al*., 1996); antioxidant activity of the flavonoids attributed to scavenging of reactive oxygen species, chelation of metal ions, and increasing the concentration of superoxide dismutase and glutathione-*S*-transferase (Smith and Luo, 2004; Shi *et al*., 2010a); antagonism of major inhibitory receptors of the central nervous system, glycine, and GABA receptors (Kondratskaya *et al*., 2002; Ivic *et al*., 2003; Strømgaard and Nakanishi, 2004; Heads *et al*., 2008); modulation of neurotransmitter concentrations or receptor densities (Diamond *et al*., 2000; Ahlemeyer and Krieglstein, 2003); reduction of NO release (Ahlemeyer and Krieglstein, 2003); inhibition of mitochondrial dysfunction (Abdel-Kader *et al*., 2007; Shi *et al*., 2010b,c); and modulation of P450 enzymes (Hellum *et al*., 2007; Rajaraman *et al*., 2009).

Diamond *et al*. (2000) reviewed the literature on the effectiveness of *Ginkgo biloba* extract and concluded that positive effects on various endpoints were observed with dosages of 120 to 300 mg per day administered for a period of 3 to 12 weeks. Standardized *Ginkgo biloba* extract is typically taken in tablet or capsule form at doses in the range of 120 to 240 mg per day. In addition to the standardized extract, ginkgo is also available as a mother tincture (ethanol extraction of fresh leaves) and as dry leaves for use in tea (Ehrlich, 2010).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Due to the complex nature of *Ginkgo biloba* extract with its many structurally diverse constituents, its precise pharmacokinetic profile remains undetermined. However, there have been many pharmacokinetics studies in humans and experimental animals that focus on key chemical classes such as the terpene trilactones and select flavonol glycosides. In general, *Ginkgo biloba* extract is well-absorbed in humans, rats, and rabbits after oral administration (Moreau *et al*., 1986; Li and Wong, 1997; Woerdenbag and van Beek, 1997).

Experimental Animals

Moreau *et al*. (1986) used radiolabeled *Ginkgo biloba* extract from leaves to assess its absorption, distribution, and elimination in male and female Sprague-Dawley rats following a single oral administration. The radiolabeled test article was obtained from ginkgo plants grown under a supply of ^{14}C-acetate. The authors demonstrated that 60% of the extract was absorbed. The pharmacokinetic results were characteristic of a two-compartment model with a half-life of about 4.5 hours. Although the labeling was nonspecific, it was found to be present mainly in the flavonol glycosides. After 72 hours, 38% of the radioactivity was excreted in exhaled CO_2, 21% in urine, and 29% in feces.

Following single oral doses of 30, 55, or 100 mg/kg *Ginkgo biloba* extract (EGb 761®) in male Sprague-Dawley rats, bilobalide and ginkgolides A and B were bioavailable with linear pharmacokinetics and half-life values of 2.2, 1.7, and 2.0 hours, respectively, for the lowest dose investigated (Biber and Koch, 1999). Ginkgolide C is difficult to detect in plasma due to matrix effects and was not included in many pharmacokinetic profiles of the terpene trilactones; however, improved methodology in a more recent pharmacokinetic study provided a ginkgolide C half-life value of 0.7 hours following intravenous administration of 8 mg/kg *Ginkgo biloba* extract in male and female Sprague-Dawley rats (Xie *et al*., 2008). Half-life values for ginkgolides A and B, and bilobalide in that study were 1.0, 1.0, and 1.1 hours, respectively. The terpene trilactones (ginkgolides A and B and bilobalide) (Ude *et al*., 2011) and major flavonoids (kaempferol, quercetin, and isorhamnetin) (Rangel-Ordóñez *et al*., 2010) were found to cross the blood brain barrier following a single or repeated oral administration of 600 mg/kg EGb 761® in male Sprague-Dawley rats.

A study assessing the metabolism of the flavonoids in rats found no intact flavonol glycosides in the blood, feces, or urine 24 hours following oral administration of *Ginkgo biloba* extract (Pietta *et al*., 1995). Metabolites identified included 3,4-dihydroxyphenylacetic acid, hippuric acid, 3-hydrocyphenylacetic acid, homovanillic acid, benzoic acid, 3-(4-hydrophenyl)propionic acid, and 3-(3-hydrophenyl)propionic acid.

Humans

In unpublished data supplied by the manufacturer (Schwabe, Karlsruche) to Kleijnen and Knipschild (1992), the bioavailabilities of ginkgolides A and B were greater than 80%, whereas that of ginkgolide C was very low following oral administration of 80 mg EGb 761®. Bioavailability of bilobalide was 70% after a dose of 120 mg EGb 761®. Half-life values of ginkgolides A and B and bilobalide were 4, 6, and 3 hours, respectively. Ginkgolides A and B and bilobalide were excreted unchanged in the urine at approximately 70%, 50%, and 30%, respectively. Mauri *et al*. (2001) observed a maximum plasma concentration of total ginkgolides (A, B, and bilobalide) of 85 and 181.8 µg/mL, respectively, following administration of 9.6 mg Ginkgoselect® and Ginkgoselect® phytosome, which were reached at 120 minutes and 180 to 240 minutes. The mean half-life values for ginkgolides A and B and bilobalide in plasma were in the range of 120 to 180 minutes for both formulations. Woelkart *et al*. (2010) conducted a study to assess pharmacokinetics of various ginkgo preparations in healthy volunteers and found similar plasma half-life values across terpene trilactones and different ginkgo preparations. Also, the areas under the plasma concentration versus time curves were comparable.

Watson and Oliveira (1999) conducted a study that measured levels of kaempferol and quercetin in urine before and after oral administration of *Ginkgo biloba* and concluded that model flavonol glycosides kaempferol and quercetin display low bioavailability and are metabolized mainly through glucuronidation. In a review of ginkgo pharmacokinetic data, Biber (2003) described the profile of the flavones as dose-linear with half-lives in the 2 to 4 hour range with rapid metabolism of parent compounds to hydroxybenzoic acids. After oral administration of *Ginkgo biloba*, Pietta *et al*. (1997) identified the flavonol metabolites in humans as 4-hydroxybenzoic acid conjugate, 4-hydroxyhippuric acid, 3-methoxy-4-hydroxyhippuric acid, 3,4-dihydrobenzoic acid, hippuric acid, and 3-methoxy-4-hydroxybenzoic acid (vanillic acid), but the phenylacetic or phenylpropionic acid metabolites observed in rats were not found in humans, suggesting that humans metabolize the flavonoids to a greater extent. Based on

areas under the plasma concentration versus time curves, it was calculated that an oral dose of 240 mg in humans corresponds roughly to an oral dose of 50 mg/kg in rats (De Feudis, 1998).

In a study conducted by Research Triangle Institute in support of the NTP *Ginkgo biloba* extract studies, *Ginkgo biloba* extract normalized to 1 or 10 µM terpene trilactones decreased human hepatic microsomal CYP2C9, CYP3A4, CYP2C8 activities *in vitro* (Etheridge *et al.*, 2007). The deglycosylation of the flavonoid glycosides by acid hydrolysis prior to incubation with the microsomes resulted in further decreases in the activities of these enzymes. Incubation of human hepatocytes with *Ginkgo biloba* extract at concentrations normalized to 10, 30, or 100 µM terpene trilactones had no effect on CYP2C8 activity. However, the hydrolyzed extract decreased the activity of CYP2C8 at 30 and 100 µM.

TOXICITY

Experimental Animals

The LD_{50} of standardized *Ginkgo biloba* extract administered orally to mice was reported to be 7.73 g/kg and the LD_{50} after intravenous administration was 1.1 g/kg for both rats and mice (Salvador, 1995).

There was no evidence of organ damage or impairment of hepatic or renal function when *Ginkgo biloba* extract was administered orally over 27 weeks to rats and mice at doses ranging from 100 to 1,600 mg/kg (Salvador, 1995). Rats exposed to EGb 761® at 4, 20, and 100 mg/kg per day for 2 years were reported to show no histopathological changes, however, details of this study are not available (Woerdenbag and De Smet, 2000).

In a chronic study conducted in Fisher 344 rats with quercetin administered by dosed feed at concentrations of 0, 1,000, 10,000, or 40,000 ppm, there were no treatment-related effects on survival, clinical signs, organ weights, or hematological and clinical chemistry parameters (Dunnick and Hailey, 1992). There were decreases in mean body weights and relative liver and kidney weights in the 40,000 ppm male and female rats.

Humans

In a review of studies of *Ginkgo biloba* extract administered to humans, Diamond *et al.* (2000) concluded that adverse events, as described in case reports, occurred in patients that were taking additional medicines or had comorbid conditions. A large randomized, double-blind clinical trial of *Ginkgo biloba* extract efficacy in reducing dementia was conducted between 2000 and 2008 (DeKosky *et al*., 2008). The authors found similar adverse event profiles between the group taking twice-daily doses of 120 mg *Ginkgo biloba* extract and the placebo group. Rates of mortality and incidences of coronary heart disease, angina, angioplasty, or stroke were also similar between the two groups.

Bent *et al*. (2005) assessed case reports and concluded a risk of spontaneous bleeding could be associated with *Ginkgo biloba* use. Kellermann and Kloft (2011) found 21 reported cases of spontaneous bleeding associated with *Ginkgo biloba* extract through 2007, with one third of the cases involving concurrent use of antiplatelet or anticoagulant therapies. The hypothesized mechanism of toxicity is that antagonism of PAF and collagen lead to inhibition of platelet aggregation (Bent *et al*., 2005). Kellermann and Kloft (2011) conducted a systematic review and meta-analysis that did not find evidence of higher bleeding risk associated with *Ginkgo biloba* extract therapy. In isolated cases, ginkgo use was associated with increased blood pressure when combined with thiazide diuretics and coma when combined with trazodone (Izzo and Ernst, 2001). Seizures occurred in two previously well-controlled epileptics within 2 weeks of beginning *Ginkgo biloba* extract supplementation and resolved after stopping supplementation (Granger, 2001).

The ginkgolic acids present in the ginkgo plant are known to cause contact dermatitis in a manner similar to that elicited by urushiols in poison ivy with limited evidence of cross-reactivity between poison ivy and ginkgo (Sowers *et al*., 1965; Tomb *et al*., 1988; Lepoittevin *et al*., 1989). For this reason, in Germany, ginkgolic acids are restricted to less than 5 ppm in standardized preparations. Two cases of allergic skin reaction (diffuse mobilliform eruptions and acute generalized excanthematous pustulosis) following oral treatment with *Ginkgo biloba* products were reported, one in the United States (Chiu *et al*., 2002) and one in Australia (Pennisi, 2006).

In reviews of the efficacy and safety of *Ginkgo biloba* extract, no serious side effects were noted in clinical trials and side effects were similar to those seen in the placebo groups (Kleijnen and Knipschild, 1992; Diamond *et al*., 2000; McKenna *et al*., 2001; Ahlemeyer and Krieglstein, 2003).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Comprehensive reproductive and developmental toxicity assessments with *Ginkgo biloba* extract were not available in the literature. Yeh *et al*. (2008) treated male Long-Evans rats with 10, 50, or 100 mg *Ginkgo biloba* extract/kg body weight for up to 28 days and found no effect on male reproductive organ weights or serum testosterone levels. Al-Yahya *et al.* (2006) reported decreased caudal epididymis and prostate weights and increased aneuploidy in testes of male Swiss albino mice treated by oral gavage with 50 or 100 mg/kg per day for 90 days and decreased rates of pregnancy and preimplantation loss in 100 mg/kg females. In a developmental study, pregnant Wistar rats were gavaged with up to 14 mg/kg *Ginkgo biloba* extract per day during the tubal transit and implantation period; no significant effects were observed in either the dams or the pups (Fernandes *et al*., 2010). In another study in pregnant rats, oral gavage with 7 or 14 mg/kg *Ginkgo biloba* extract on gestational days 8 to 20 did result in significant decreases in fetal weight (Pinto *et al*., 2007).

Humans

No studies on the reproductive or developmental toxicity of *Ginkgo biloba* extract in humans were found in the literature.

CARCINOGENICITY

Experimental Animals

No chronic toxicity studies with *Ginkgo biloba* were identified in the literature. However, quercetin, one of the major flavonol glycosides of ginkgo, has been assessed for carcinogenicity in rats, mice, and hamsters (reviewed in Harwood *et al.*, 2007). In the NTP 2-year quercetin bioassay in male and female F344/N rats, the significant finding was an increased incidence of renal tubule cell adenoma in male rats (Dunnick and Hailey, 1992). In a reevaluation

of the kidney neoplasms and renal histopathology from that study, Hard *et al*. (2007) determined that the responsible mechanism of carcinogenicity was chronic progressive nephropathy. Hirono *et al*. (1981) conducted carcinogenicity studies with the flavonol glycosides quercetin or rutin using ACI rats given dosed feed for a period of 540 or 850 days and did not find significant increases in neoplasm incidence with either chemical.

Studies exploring the carcinogenic activity of *Ginkgo biloba* in chemically-induced cancer models have been conducted. In all cases, *Ginkgo biloba* decreased the severity of chemical-induced cancer development (Agha *et al*., 2001; Dias *et al*., 2008; Jiang *et al*., 2009).

Humans

Two epidemiological studies explored carcinogenicity associated with use of *Ginkgo biloba* supplements. In a population-based, case-control study reported by Ye *et al.* (2007), in which the case group included 668 women in Massachusetts and New Hampshire diagnosed with epithelial ovarian cancer matched to 721 women in the control group, an inverse association (OR=0.41; 95% confidence interval, 0.20-0.84; P=0.01) was found between *Ginkgo biloba* use and risk for ovarian cancer. A more recent study by Biggs *et al*. (2010) used data from the largest epidemiological study of *Ginkgo biloba* efficacy (Ginkgo Evaluation of Memory Study) conducted to date to analyze cancer as a secondary endpoint. The study population consisted of 3,069 participants, age 75 years or greater, that were randomly assigned to receive twice daily doses of either a placebo or *Ginkgo biloba* extract (120 mg EGb 761®) and were followed for approximately 6 years. Researchers found an increased risk of breast (hazard ratio, 2.15; 95% confidence interval, 0.97-4.80; P=0.06) and colorectal (hazard ratio, 1.62; 95% confidence interval, 0.92-2.87; P=0.10) cancers and a decreased risk of prostate cancer (hazard ratio, 0.71; 95% confidence interval, 0.43-0.17; P=0.08) in the population receiving *Ginkgo biloba* extract.

GENETIC TOXICITY

Ginkgo biloba Extract

The genetic toxicity literature on *Ginkgo biloba* is primarily focused on its antimutagenic effects in experiments using cotreatment with known genotoxicants. However, quercetin, one of the principal components found in *Ginkgo biloba* extracts, was reported to induce genetic damage, as measured by a number of different endpoints.

Ginkgo biloba extract (EGb 761®), over a concentration range of 5 to 100 µg/mL, reduced the mutagenicity of 15 µg/mL ofloxacin and 5 µg/mL acridine orange in *Euglena gracilis* in a dose-dependent manner (Krizková *et al*. 2008); the mutagenicity of both agents was reduced by 99% at the highest dose of *Ginkgo biloba* extract. From additional experiments designed to elucidate the mechanisms of *Ginkgo biloba* extract antimutagenicity, the authors concluded that *Ginkgo biloba* extract was an effective antioxidant and it bound directly to acridine orange, inhibiting its ability to interact with DNA.

Significant dose-related reductions in genotoxicant-induced micronuclei were seen in reticulocytes of mice following co-administration of *Ginkgo biloba* extract and either cyclophosphamide or mitomycin C (Vilar *et al*., 2009). In another experiment, the bacterial mutagenicity of tobacco smoke condensates from cigarettes was significantly reduced when *Ginkgo biloba* leaf extract was added to the cigarette filters (Wang *et al*., 2010). When rats were exposed for 30 days (twice daily) to the smoke from these cigarettes containing *Ginkgo biloba* leaf extract, the frequency of micronucleated reticulocytes was reported to be lower than the frequency in rats exposed to smoke from control cigarettes containing tobacco but no *Ginkgo biloba* (Wang *et al*., 2010). However, the experimental details provided in this published study were insufficient to independently evaluate the data.

Gingko biloba-containing tablet supplements were shown to reduce the frequencies of micronucleated lymphocytes over time in radioiodine-treated Graves' disease patients, without adversely affecting clinical outcome (Dardano *et al*., 2007). Similarly, in human lymphocytes *in vitro*, pretreatment with concentrations of 50 µg/mL and higher of *Ginkgo biloba* was shown to reduce the frequency of chromosomally aberrant cells induced by exposure to 10 Gy radiation (Emerit *et al*., 1995).

Although there is no evidence of genotoxicity from the limited number of studies conducted with *Ginkgo biloba*, ginkgolic acids, which are components of *Ginkgo biloba*, were reported to induce DNA damage as measured by the comet assay in primary rat hepatocytes *in vitro* (Westendorf and Regan, 2000).

Quercetin

Quercetin, a flavonol, was identified in the *Ginkgo biloba* extract used in the NTP 2-year bioassay. There is a large body of literature on the genetic toxicity of quercetin, and it was the subject of an NTP Technical Report (NTP, 1992). A summary of the extensive genetic toxicology literature for quercetin follows.

Quercetin induced DNA strand breaks, measured by alkaline single cell gel electrophoresis, in HepG2 cells, HeLa cells, and human lymphocytes *in vitro* (Duthie *et al*., 1997). It was shown to be mutagenic in several strains of *Salmonella typhimurium*, with and without S9 (Bjeldanes and Chang, 1977; Sugimura *et al*., 1977; Hardigree and Epler, 1978; MacGregor and Jurd, 1978; Brown and Dietrich, 1979; Nagao *et al*., 1981; Stoewsand *et al*., 1984; Crebelli *et al*., 1987; Zeiger *et al*., 1992).

Quercetin induced micronuclei in V79 cells and human lymphocytes *in vitro*, with and without S9 (Caria *et al*., 1995). The micronuclei observed in human lymphocytes were kinetochore-negative, suggesting they arose from chromosomal breakage events. Consistent with this mode of action, quercetin was reported to induce chromosomal aberrations in V79 cells, CHO-AT3-2 cells, Don-6 cells, and Chinese hamster ovary (CHO) cells with and without S9 (Yoshida *et al*., 1980; Carver *et al*., 1983; Kubiak and Rudek, 1990; NTP, 1992; Gaspar *et al*., 1994).

Quercetin was also positive in tests for induction of sister chromatid exchanges in cultured Chinese hamster Don-6 cells, B-131 cells, and ovary cells and in cultured human fibroblast HE2144 cells and human lymphocytes (Yoshida *et al*., 1980; Kubiak and Rudek, 1990; NTP, 1992).

Although quercetin has clearly been shown to induce cytogenetic damage *in vitro*, it did not induce a consistent or significant increase in gene mutations at the *hgprt*, *aprt*, or ATPase loci in CHO cells, V79 cells, or mouse lymphoma L5178Y cells *in vitro* (Carver *et al*., 1983; Van der Hoeven *et al*., 1984). However, Maruta *et al*. (1979)

reported that quercetin induced 8-azaguanine resistance in V79 cells with and without metabolic activation, and several laboratories demonstrated induction of trifluorothymidine resistance in L5178Y$^{tk+/-}$ mouse lymphoma cells (Amacher *et al*., 1980; Meltz and MacGregor 1981; Van der Hoeven *et al*., 1984). Quercetin also induced dose-related increases in the frequencies of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* administered the compound by adult feeding at doses of 8.25 and 16.5 × 10^{-2} M (Watson, 1982).

In contrast to the cytogenetic damage seen with quercetin in mammalian cells *in vitro*, results of *in vivo* mouse bone marrow micronucleus tests with quercetin were negative (Aeschbacher *et al*., 1982; NTP, 1992; Caria *et al*., 1995).

Additional Mutagenic Compounds in *Gingko biloba* Extract

Kaempferol

The mutagenicity of kaempferol was investigated in a number of standard *in vitro* assays. The compound was mutagenic in *S. typhimurium* with S9 (Nagao *et al*., 1981; Silva *et al*., 1997) and produced dose-dependent increases in chromosomal aberrations and micronuclei in V79 cells, with and without S9 (Silva *et al*., 1997). Kaempferol (10 to 500 µM) induced DNA damage, as measured by the comet assay, in human lymphocytes treated *in vitro* (Anderson *et al*., 1997), but when co-administered with the known food mutagens Trp or IQ, kaempferol reduced the levels of DNA damage induced by the food mutagens alone (Anderson *et al*., 1997). Kaempferol also induced dose-related increases in the frequencies of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* administered the compound by adult feeding at doses of 8.7 and 17.4 × 10^{-2} M (Watson, 1982).

Rutin

Rutin was shown to reduce the mutagenic effects of known genotoxicants in *S. typhimurium* strain TA102 (Edenharder and Grunhage, 2003). Rutin (100 to 500 µM) induced DNA damage, as measured by the comet assay, in human lymphocytes treated *in vitro*, but when co-administered at high concentrations (250 to 500 µM) with the known food mutagens Trp or IQ, rutin reduced the levels of DNA damage induced by the food mutagens alone (Anderson *et al*., 1997).

STUDY RATIONALE

Ginkgo biloba extract was nominated for study by the National Institute of Environmental Health Sciences because of its widespread use as an herbal supplement to promote mental function and limited availability of toxicity and carcinogenicity data. Additionally, one of the major ingredients in *Ginkgo biloba* extract, quercetin, is a known mutagen. Oral gavage was chosen as the route most relevant to human exposure through ingestion of *Ginkgo biloba* extract supplements. The test article selection was based on availability of bulk product and market share of the manufacturer at the study initiation.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Ginkgo biloba Extract

The original intent was to use standardized extract EGb 761®, manufactured by Wilhelm Schwabe, due to its use in many human studies. However, this material was not available to the NTP because unformulated EGb 761® was exclusively sold to pharmaceutical companies at the time of procurement for the NTP studies. Through industry contacts, the NTP learned that Shanghai Xing Ling Science and Technology Pharmaceutical Company (Shanghai, China) produced an extract similar to the Schwabe extract that was widely distributed in commerce (personal communication). *Ginkgo biloba* extract from leaves was obtained from Shanghai Xing Ling Science and Technology Pharmaceutical Company, Ltd., in two lots (020703 and GBE-50-001003). Lot 020703 was used during the 3-month and 2-year studies. Lot GBE-50-001003 was used only for methods development. Identity, purity, stability, and moisture analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO); in addition, the study laboratory at Battelle Columbus Operations (Columbus, OH) confirmed the identity of the test article by infrared spectroscopy (Appendix I). Reports on analyses performed in support of the *Ginkgo biloba* extract studies are on file at the National Institute of Environmental Health Sciences.

The identity and purity of the test article were determined by using a combination of techniques and authentic standards specific for ginkgo. Estimation of the distribution of particle sizes in lot GBE-50-001003 was determined using a sieve method, and the results indicated that 84.3% of the test article passed through a No. 140 (106 µm) sieve (Table I1). The bulk density of lot 020703 was determined to be 0.521 g/mL. To evaluate organic constituents of the test article, extracts were prepared, and a combination of chromatographic and spectrometric techniques was used to characterize this lot. High-performance thin layer chromatography (HPTLC) fingerprint analyses were conducted for ginkgolides and flavonoids in methanol extracts of the test article using two systems specified in Application Notes F-16A and F-16B, respectively, obtained from CAMAG Scientific, Inc. (Wilmington, NC). Many components of the test article were tentatively identified using various chromatography/mass

spectrometry techniques, including liquid chromatography/mass spectrometry, gas chromatography/mass spectrometry, and matrix-assisted laser-desorption ionization time-of-flight mass spectrometry. High-performance liquid chromatography (HPLC) was used to profile ethanol:water:12N hydrochloric acid (64:26:10) extracts of the bulk material and to quantitate (using caffeine as an internal standard) α-glycosides and terpenoids in these extracts. Analyses were conducted on nonhydrolyzed extracts and also on extracts hydrolyzed at 90° C for at least 2 hours, utilizing commercially obtained standards with matching retention times published in the literature (Hasler *et al*., 1992). For these assays, methanol:water (50:50) extracts of the *Ginkgo biloba* powder were partitioned with dichloromethane and dried over anhydrous sodium sulfate. The residue was reconstituted with methanol and analyzed using total ion current and single ion response mode following the methodology of Ndjoko *et al*. (2000) and Li *et al*. (2002). Further information on these methods can be found in Gray *et al*. (2005, 2007).

As determined by weight loss on drying, moisture content for lot 020703 was 0.90%. Developed, dried HPTLC plates were viewed under UV light, and multiple bands were obtained for the test article by both systems; bands consistent with those for standards obtained from Sigma-Aldrich (St. Louis, MO) of the ginkgolides bilobalide, ginkgolide A, and ginkgolide B, and of the flavonoid rutin hydrate were observed. In HPLC/UV profiles of the test article extracts, 37 components were observed to have peak areas greater than or equal to 0.05% of the total peak area, and three components were identified: quercetin, kaempferol, and isorhamnetin, which had peak areas equal to 34.08%, 27.77%, and 5.43%, respectively, of the total peak area. In HPLC/ELS profiles of the test article extracts, 18 components were observed to have peak areas greater than or equal to 0.05% of the total peak area, and seven components were identified: bilobalide, ginkgolide C, ginkgolide A, ginkgolide B, quercetin, kaempferol, and isorhamnetin, which had peak areas equal to 17.30%, 3.25%, 9.06%, 2.05%, 28.74%, 12.58%, and 2.24%, respectively, of the total peak area. Quantitation assays of α-glycosides in the hydrolyzed extracts using HPLC/UV indicated that the test material contained 16.71% quercetin, 12.20% kaempferol, and 2.37% isorhamnetin. HPLC/ELS quantitation assays of terpenoids in the hydrolyzed extracts determined that the test material contained 6.94% bilobalide, 3.06% ginkgolide C, 3.74% ginkgolide A, and 1.62% ginkgolide B. HPLC/MS analyses for the presence of ginkgolic acids I and II using standards from ChromaDex, Inc. (Irvine, CA), and for colchicine using the colchicine standard from Sigma-Aldrich. The concentration of total ginkgolic acids was 10.45 ± 2.40 ppm. No observable peaks for colchicine were observed in the test material.

Stability studies of lot 020703 of the bulk chemical were performed by the analytical chemistry laboratory using HPLC analyses by the same UV and ELS detection techniques used to characterize this lot of the bulk product. Results of these assays were inconclusive due to high amounts of variability in the measurements, but the seven α-glycosides and terpenoid components in lot 020703 appeared to be stable, within experimental error, at temperatures up to approximately 60° C when the bulk material was stored protected from light. To ensure stability, the bulk chemical was stored at room temperature in amber glass bottles. Periodic reanalyses of the bulk chemical were performed during the 3-month and 2-year studies by the study laboratory, and stability was monitored using an isocratic HPLC/UV system. No degradation of the bulk chemical was detected.

Corn Oil

NF-grade corn oil was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA) and was used as the vehicle in the 3-month and 2-year studies. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than the rejection level of 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared three times during the 3-month studies and approximately every 4 weeks during the 2-year studies by mixing *Ginkgo biloba* extract with corn oil (Table I2). Homogeneity studies of the 25 and 400 mg/mL dose formulations were performed by the study laboratory using an isocratic HPLC/UV system; this system was also used by Battelle Chemistry Support Services (Columbus, OH) to perform stability studies of a 1 mg/mL formulation made with lot GBE-50-001003 of *Ginkgo biloba* extract. Homogeneity was confirmed, and stability was confirmed for at least 42 days for formulations stored in sealed plastic bottles protected from light at room temperature or at approximately 5° C, and for at least 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of *Ginkgo biloba* extract were conducted by the study laboratory using the isocratic HPLC/UV system. During the 3-month studies, the dose formulations were analyzed three times; all 15 dose formulations for rats and mice were within 10% of the target concentrations (Table I3). Animal room samples of these dose formulations were also analyzed; all 15 animal room samples for rats and all 15 for mice were

within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 12 weeks; animal room samples were also analyzed (Table I4). All 30 dose formulations analyzed for rats and mice were within 10% of the target concentrations; all 12 animal room samples for rats and all 12 for mice were within 10% of the target concentrations

3-MONTH STUDIES

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to *Ginkgo biloba* extract and to determine the appropriate doses to be used in the 2-year studies. The high doses for the 3-month studies were based on the limits of gavageability and homogeneity of the substance.

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Rats were quarantined for 11 (males) or 12 (females) days; mice were quarantined for 13 (females) or 14 (males) days. Rats and mice were 6 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At 1 month and 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 received *Ginkgo biloba* extract in corn oil by gavage at doses of 0, 62.5 (rats only), 125, 250, 500, 1,000, or 2,000 (mice only) mg/kg 5 days per week for 14 weeks. Vehicle control animals received the corn oil vehicle alone; dosing volumes were 2.5 mL/kg for rats and 5 mL/kg for mice. Groups of 10 male and 10 female clinical pathology rats received the same doses for 23 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings were recorded initially, weekly thereafter, and at the end of the studies for core study rats and mice. Core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Blood was collected from clinical pathology study rats on days 4 and 23 and from core study rats at the end of the study for hematology and clinical chemistry analyses; blood was collected at the end of the study from all mice for hematology analyses. All animals were anesthetized with a carbon dioxide/oxygen mixture and blood was drawn from the retroorbital plexus (rats) or retroorbital sinus (mice). Whole blood samples for hematology were placed into tubes containing EDTA; clinical chemistry samples were placed into serum collection tubes. Hematology determinations were performed on an ADVIA 120 Hematology system (Bayer Diagnostics, Tarrytown, NY). Clinical chemistry analyses were performed on a Hitachi 911 (Boehringer Mannheim, Indianapolis, IN) using reagents supplied by the manufacturer. The parameters measured are listed in Table 2.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats in the 0, 250, 500, and 1,000 mg/kg groups and mice in the 0, 500, 1,000, and 2,000 mg/kg groups. The parameters evaluated are listed in Table 2. For 12 consecutive days prior to scheduled terminal kill, 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 count and motility. The left testis and left 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 left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all core study vehicle control rats and mice, 1,000 mg/kg rats, and 2,000 mg/kg mice; the liver and the thyroid gland of rats and the liver of mice were examined in the remaining groups, and the kidney was examined in the remaining groups of male mice (except 125 mg/kg). Table 2 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, NTP pathologist, reviewing pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice received *Ginkgo biloba* extract in corn oil by gavage at doses of 100, 300, or 1,000 mg/kg (rats), or 200, 600, or 2,000 mg/kg (mice) 5 days per week for 104 or 105 (female rats) weeks. Vehicle control animals received the corn oil vehicle alone; the dosing volumes were 2.5 mL/kg for rats and 5 mL/kg for mice. Groups of 10 male and 10 female special study rats received the same doses for 14 weeks.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats were quarantined for 13 (males) or 14 (females) days and mice were quarantined for 14 (males) or 15 (females) days before the beginning of the studies. Five male and five female rats and mice were

randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 6 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program using extra rats and mice at 1 month, sentinel rats and mice at 6, 12, and 18 months, and 1,000 mg/kg rats and 2,000 mg/kg mice at study termination; five males and five females were used at each time point (Appendix K).

Animal Maintenance

All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the Battelle Columbus Operations Animal Care and Use Committee and conducted in accordance with all relevant NTP animal care and use policies and applicable federal, state, and local regulations and guideline. Rats were housed two or three (core study males) or five (special study males and females and core study females) per cage and mice were housed individually (males) or five per cage (females). Feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Body weights were recorded initially, weekly for the first 13 weeks, every 4 weeks thereafter and at study termination. Clinical findings for core study rats and mice were recorded during study week 5, every 4 weeks thereafter, and at study termination.

Blood was taken from the retroorbital plexus of special study rats on day 22 and at week 14 and processed into serum for thyroid hormone determinations. Radioimmunoassays were performed for thyroid stimulating hormone and total triiodothyronine using a Packard Cobra II gamma counter (Packard Instrument Company, Meriden, CT). Assays for total thyroxine were performed on a Hitachi 911 Chemistry Analyzer (Boehringer Mannheim, Indianapolis, IN) using a Roche Diagnostics (Indianapolis, IN) enzyme immunoassay test system.

For special study rats at 14 weeks, the liver and thyroid gland were weighed and examined microscopically. Complete necropsies and microscopic examinations were performed on all core study 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 (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. 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 evaluated slides from all tumors and all potential target organs, which included the kidney and liver of rats and mice; the adrenal cortex, nose, and thyroid gland of rats; the bone marrow, parathyroid gland, spleen, and glandular stomach of male rats; the pancreas, pituitary gland, and uterus of female rats; and the thymus of female mice.

The quality assessment report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator 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. 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 2
Experimental Design and Materials and Methods in the Gavage Studies of *Ginkgo biloba* Extract

3-Month Studies	2-Year Studies
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species	
F344/N rats B6C3F1/N mice	F344/N rats B6C3F1/N mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days	Rats: 13 (males) or 14 (females) days Mice: 14 (males) or 15 (females) days
Age When Studies Began	
6 to 7 weeks	6 to 7 weeks
Date of First Dose	
Rats: February 9, 2004 (males) February 10, 2004 (females) Mice: February 12, 2004 (males) February 11, 2004 (females)	Rats: March 23, 2005 (males) March 24, 2005 (females) Mice: March 17, 2005 (males) March 18, 2005 (females)
Duration of Dosing	
5 days/week for 14 weeks	5 days/week for 104 or 105 (female rats) weeks
Date of Last Dose	
Rats: May 10, 2004 (core males) May 11, 2004 (core females) March 2, 2004 (clinical pathology study males) March 3, 2004 (clinical pathology study females) Mice: May 13, 2004 (males) May 12, 2004 (females)	Rats: March 20, 2007 (core males) March 22, 2007 (core females) June 21, 2005 (special study males) June 22, 2005 (special study females) Mice: March 12, 2007 (males) March 15, 2007 (females)
Necropsy Dates	
Rats: May 11, 2004 (males) May 12, 2004 (females) Mice: May 14, 2004 (males) May 13, 2004 (females)	Rats: March 19-21, 2007 (males) March 21-23, 2007 (females) Mice: March 12-13, 2007 (males) March 14-16, 2007 (females)
Age at Necropsy	
Mice: 19 to 20 weeks	Rats: 110 to 111 weeks Mice: 109 to 111 weeks
Size of Study Groups	
10 males and 10 females	Core study rats and mice: 50 males and 50 females Special study rats: 10 males and 10 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of *Ginkgo biloba* Extract

3-Month Studies	2-Year Studies
Animals per Cage Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 2 or 3 (core study males) or 5 (special study males and all females) Mice: 1 (males) or 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo
Diet Irradiated NTP-2000 wafer diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed at least weekly	Same as 3-month studies
Water Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 3-month studies
Cages Polycarbonate (Lab Products, Inc., Seaford, DE), changed once weekly for individually housed animals and twice weekly for group housed animals	Same as 3-month studies
Bedding Irradiated Sani-Chips® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed once weekly for individually housed animals and twice weekly for group housed animals	Same as 3-month studies
Rack Filters Spun bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 3-month studies
Racks Stainless steel drawer type (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks	Same as 3-month studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses Rats: 0, 62.5, 125, 250, 500, or 1,000 mg/kg in corn oil (dosing volume 2.5 mL/kg) Mice: 0, 125, 250, 500, 1,000, or 2,000 mg/kg in corn oil (dosing volume 5 mL/kg)	Rats: 0, 100, 300, or 1,000 mg/kg in corn oil (dosing volume 2.5 mL/kg) Mice: 0, 200, 600, or 2,000 mg/kg in corn oil (dosing volume 5 mL/kg)
Type and Frequency of Observation Observed twice daily; core study animals were weighed initially, weekly thereafter, and at the end of the studies; clinical findings (core animals) were recorded initially, weekly thereafter, and at the end of the studies.	Observed twice daily; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies; clinical findings for core study animals were recorded week 5, monthly thereafter, and at the end of the studies.
Method of Kill Carbon dioxide asphyxiation	Same as 3-month studies
Necropsy Necropsies were performed on all core study rats and mice. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study rats and mice. The liver and thyroid gland of special study rats were weighed.

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of *Ginkgo biloba* Extract

3-Month Studies	2-Year Studies
Clinical Pathology Blood was collected from the retroorbital plexus (rats) or retroorbital sinus (mice). Clinical pathology rats were bled on days 4 and 23 and core study animals were bled at the end of the studies for hematology and clinical chemistry (rats only). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile salts	Blood was collected from the retroorbital plexus of special study rats at day 22 and at week 14 for thyroid hormone analyses. ***Thyroid hormones***: thyroid stimulating hormone, triiodothyronine, and thyroxine
Histopathology Complete histopathology was performed on all core study vehicle control rats and mice, 1,000 mg/kg rats, and 2,000 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the liver and thyroid gland of rats, the liver of mice, and the kidney of male mice (except 125 mg/kg) were examined in the remaining core study groups.	Complete histopathology was performed on all core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The liver and thyroid gland were examined in special study rats.
Sperm Motility and Vaginal Cytology At the end of the studies, spermatid and sperm samples were collected from male rats in the 0, 250, 500, and 1,000 mg/kg groups and male mice in the 0, 500, 1,000, and 2,000 mg/kg groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from female rats in the 0, 250, 500, and 1,000 mg/kg groups and female mice in the 0, 500, 1,000, and 2,000 mg/kg groups. The proportion of regularly cycling females, estrous cycle length, and probability of extended or skipped estrous cycle stages were evaluated.	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; 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 are presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) 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., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. 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 A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal kill.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total

number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal kill; if the animal died prior to terminal kill and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, thyroid hormone, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) 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 (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Proportions of regular cycling females in each dosed group were compared to the control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

QUALITY ASSURANCE METHODS

The 3-month 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 covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. 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, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of *Ginkgo biloba* extract was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies (Witt *et al.*, 2000). 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

3-MONTH STUDY

All rats survived to the end of the study; final mean body weights and mean body weight gains of all dosed groups were similar to those of the vehicle controls (Table 3 and Figure 2). No chemical-related clinical findings were observed.

On day 23 and at week 14, there were mild increases in platelet counts in the 500 and 1,000 mg/kg males; at week 14, there was an increase in reticulocyte counts in the 250 mg/kg or greater male groups (Table F1). The

TABLE 3
Survival and Body Weights of Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	100 ± 4	322 ± 5	222 ± 4	
62.5	10/10	100 ± 4	329 ± 5	229 ± 4	102
125	10/10	99 ± 4	322 ± 3	222 ± 4	100
250	10/10	100 ± 4	326 ± 4	227 ± 3	101
500	10/10	99 ± 4	324 ± 2	225 ± 4	101
1,000	10/10	99 ± 4	314 ± 5	215 ± 5	98
Female					
0	10/10	91 ± 3	192 ± 2	101 ± 3	
62.5	10/10	91 ± 3	191 ± 2	100 ± 4	99
125	10/10	90 ± 3	194 ± 3	104 ± 3	101
250	10/10	90 ± 3	194 ± 4	104 ± 4	101
500	10/10	91 ± 2	193 ± 1	102 ± 3	100
1,000	10/10	90 ± 3	193 ± 2	103 ± 2	101

[a] Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.
[b] Number of animals surviving at 14 weeks/number initially in group

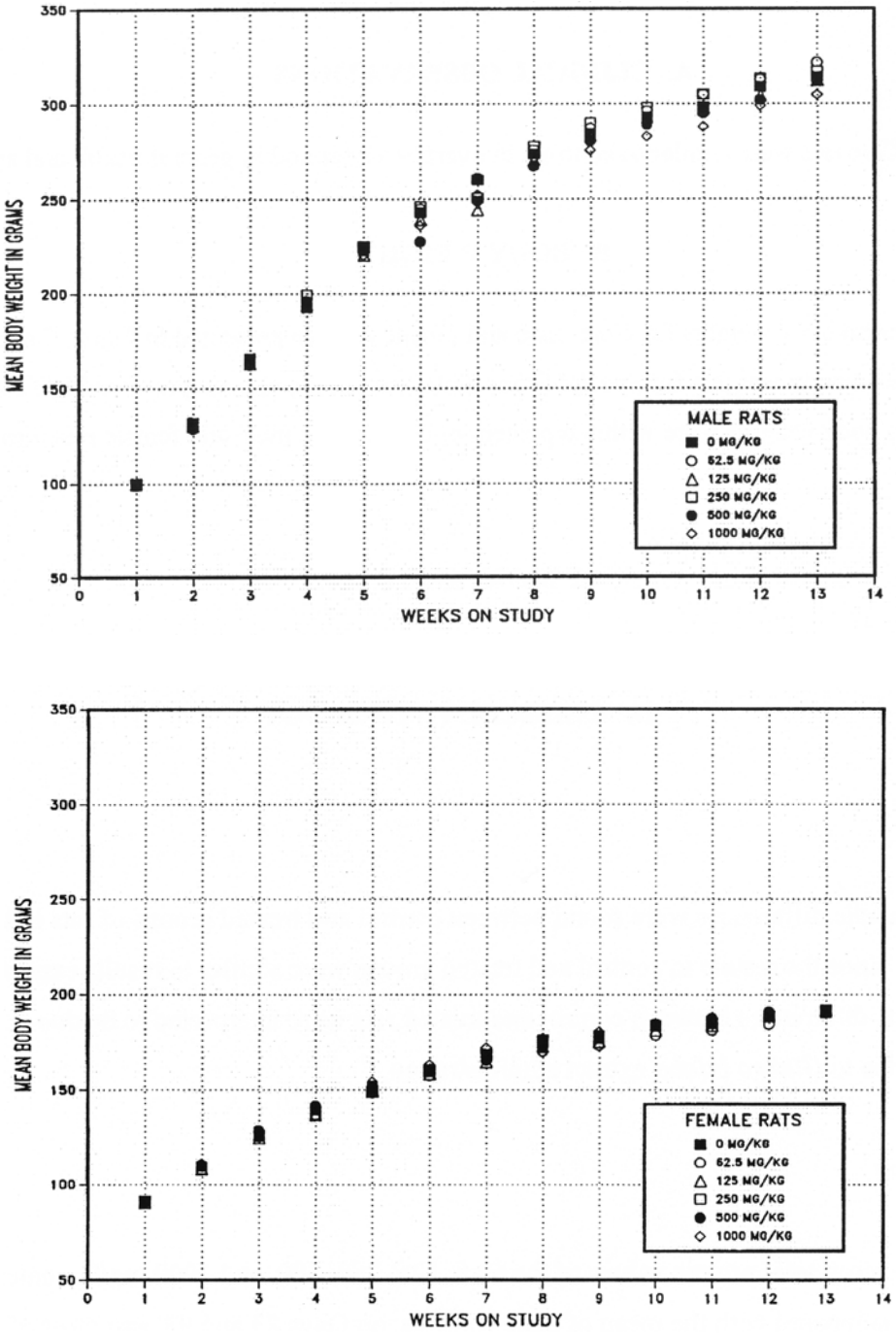


FIGURE 2
Growth Curves for Rats Administered *Ginkgo biloba* Extract by Gavage for 3 Months

reticulocyte count increases were not accompanied by changes in other erythrocyte indices. The increases may reflect increased production or altered peripheral distribution.

On day 23, there were mild increases (up to 9%) in serum albumin and total protein concentrations in all dosed male groups, with similar increases in the 125 mg/kg or greater male groups at week 14 (Tables 4 and F1). These increases generally occurred in a dose-related manner. Paired increases in serum albumin and total protein concentrations suggest dehydration.

Decreases in serum bile salt concentration occurred on day 4 in the 125 mg/kg or greater male groups and at week 14 in the 250 mg/kg or greater males and the 125 mg/kg or greater females (Tables 4 and F1). These decreases may represent disruption of enterohepatic circulation of bile salts (Tolman and Rej, 1999).

At week 14, there were mild (up to 45%) treatment-related decreases in serum activity of alanine aminotransferase in all dosed groups except 62.5 mg/kg females (Tables 4 and F1). The significance and mechanism of the decreased activity are unknown but may indicate decreased hepatocellular enzyme production or release, or enzyme assay interference. In particular, decreases have been associated with perturbations of gluconeogenesis or administration of substances that inhibit the cofactor pyridoxal phosphate (Hall, 2007; Evans, 2009).

On day 23 and at week 14, alkaline phosphatase activities in most groups of dosed males and all groups of dosed females were significantly decreased (Tables 4 and F1). In rats, circulating serum alkaline phosphatase activity is primarily of intestinal or bone origin. While the mechanism in the current study is not known, decreased feed consumption due to toxicity or poor palatability has been associated with decreased alkaline phosphatase activity (Hall, 2007; Evans, 2009). However, body weights were unaffected in the current study, indicating that feed consumption was not reduced.

Absolute and relative liver weights of all dosed groups of males and females were significantly increased compared to those of the vehicle control groups (Table G1).

TABLE 4
Selected Clinical Chemistry Data for Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Total protein (g/dL)						
Day 4	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1
Day 23	6.5 ± 0.0	6.7 ± 0.1**	6.8 ± 0.1**	6.7 ± 0.1**	6.8 ± 0.1**	7.0 ± 0.1**
Week 14	6.6 ± 0.0	6.8 ± 0.1	6.9 ± 0.1**	7.0 ± 0.1**	7.2 ± 0.1**	7.2 ± 0.1**
Albumin (g/dL)						
Day 4	4.0 ± 0.0	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.0	4.0 ± 0.1	4.0 ± 0.1
Day 23	4.4 ± 0.0	4.5 ± 0.0*	4.5 ± 0.0*	4.4 ± 0.0*	4.5 ± 0.1*	4.6 ± 0.0**
Week 14	4.6 ± 0.0	4.7 ± 0.0	4.8 ± 0.0*	4.8 ± 0.0**	4.9 ± 0.0**	4.9 ± 0.0**
Alanine aminotransferase (IU/L)						
Day 4	64 ± 2	66 ± 3	65 ± 2	64 ± 2	64 ± 2	65 ± 2
Day 23	57 ± 1	62 ± 2	64 ± 4	56 ± 1	58 ± 2	58 ± 3
Week 14	74 ± 6	41 ± 1**	45 ± 2**	42 ± 1**	45 ± 1**	42 ± 1**
Alkaline phosphatase (IU/L)						
Day 4	707 ± 11	699 ± 14	678 ± 17	701 ± 14	699 ± 22	697 ± 13
Day 23	482 ± 6	468 ± 12	464 ± 10	451 ± 13*	450 ± 11*	446 ± 7**
Week 14	224 ± 6	210 ± 5	200 ± 3**	188 ± 3**	194 ± 5**	181 ± 3**
Bile salts (µmol/L)						
Day 4	9.6 ± 1.0	7.3 ± 0.8	5.9 ± 0.8*	7.0 ± 0.8*	5.5 ± 1.2**	5.3 ± 0.5**
Day 23	4.5 ± 0.9	2.7 ± 0.4	3.9 ± 0.6	4.4 ± 0.7	2.7 ± 0.2	2.9 ± 0.4
Week 14	5.5 ± 0.8	3.8 ± 0.4	3.9 ± 0.5	3.3 ± 0.4**	3.8 ± 0.7*	2.8 ± 0.4**
Female						
n	10	10	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 4	54 ± 2	54 ± 2	53 ± 2	57 ± 2	53 ± 2	52 ± 2
Day 23	40 ± 1	44 ± 1*	41 ± 1	43 ± 1	40 ± 1	41 ± 2
Week 14	49 ± 3	45 ± 1	40 ± 1**	39 ± 1**	39 ± 1**	35 ± 1**
Alkaline phosphatase (IU/L)						
Day 4	563 ± 10	536 ± 19	518 ± 10	546 ± 14	517 ± 18	519 ± 20
Day 23	365 ± 5	348 ± 11*	340 ± 9*	339 ± 8*	320 ± 8**	311 ± 9**
Week 14	183 ± 7	160 ± 7*	159 ± 4*	138 ± 4**	146 ± 4**	135 ± 3**
Bile salts (µmol/L)						
Day 4	6.1 ± 1.1	5.8 ± 0.8	3.7 ± 0.4	5.0 ± 0.7	3.6 ± 0.4	3.5 ± 0.4
Day 23	5.6 ± 0.9	6.4 ± 0.9	4.9 ± 1.3	5.0 ± 0.8	5.3 ± 1.0	3.3 ± 0.5
Week 14	10.0 ± 1.2	8.7 ± 0.9	6.0 ± 0.9*	4.3 ± 0.5**	3.4 ± 0.5**	3.1 ± 0.4**

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Other than a slight (11%) decrease in spermatid heads/mg testis in males in the 250 mg/kg group, there were no significant differences in sperm parameters of male rats or in the estrous cycle of female rats treated with 250, 500, or 1,000 mg/kg when compared to the vehicle controls (Tables H1 and H2). The minimal, non-dose-related decrease in relative spermatid counts, in the absence of any other concordant absolute count or histologic effects, was not considered to be related to administration of *Ginkgo biloba* extract.

The incidences of hepatocyte hypertrophy in all dosed groups of males and in 500 and 1,000 mg/kg females were significantly greater than those in the vehicle control groups (Table 5). Hepatocyte fatty change occurred in all dosed males. The hypertrophy began in the centrilobular areas and expanded to the midzonal areas with a dose-related increase in severity from minimal to moderate in the males and minimal severity in the females. Microscopically, hepatocytic hypertrophy was characterized by an enlargement of the hepatocytes up to approximately one and a half times the normal size. The hypertrophic hepatocytes had increased amounts of cytoplasm and slightly enlarged nuclei. Fatty change consisted of small numbers of scattered midzonal hepatocytes having small to a few large, clear, discrete intracytoplasmic vacuoles that had the typical appearance of lipid droplets filling most or all of the cytoplasm.

The incidences of thyroid gland follicular cell hypertrophy were significantly increased in 500 and 1,000 mg/kg males and in 1,000 mg/kg females (Table 5). The hypertrophy was minimal to mild in severity in males and minimal in females and was characterized by an increase in the size of the follicular cells with the affected cells often assuming a tall cuboidal to low columnar shape. Sometimes enlarged follicular cells formed small protrusions into the lumen, and often there was an increase in clear vacuolation (resorption vacuoles) in the follicular cell cytoplasm due to increased resorptions of colloid. Follicles at the periphery of the vehicle control thyroid glands commonly were dilated and lined with flattened follicular epithelial cells, indicating the follicles were relatively inactive, while peripheral follicles in the affected thyroid glands were usually lined by large cuboidal cells, indicating the follicles were still somewhat active.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
Liver[a]	10	10	10	10	10	10
Hepatocyte, Hypertrophy[b]	0	10** (1.0)[c]	10** (1.2)	10** (1.7)	10** (2.2)	10** (3.0)
Hepatocyte, Fatty Change	0	10** (1.4)	10** (1.7)	10** (1.8)	10** (1.7)	10** (1.3)
Thyroid Gland	10	10	10	10	10	10
Follicular Cell, Hypertrophy	0	2 (1.0)	0	3 (1.3)	10** (1.3)	10** (2.0)
Nose	10	10	10	10	10	10
Olfactory Epithelium, Pigmentation	0	0	0	0	5* (1.0)	7** (1.0)
Female						
Liver	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	1 (1.0)	0	0	9** (1.2)	10** (1.1)
Thyroid Gland	10	10	10	10	10	10
Follicular Cell, Hypertrophy	0	0	0	0	3 (1.0)	5* (1.0)
Nose	10	10	10	10	10	10
Olfactory Epithelium, Pigmentation	0	0	5* (1.0)	5* (1.0)	9** (1.2)	7** (1.6)
Olfactory Epithelium, Atrophy	0	0	1 (1.0)	0	3 (1.3)	3 (2.3)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

The incidences of pigmentation in the olfactory epithelium of the nose were significantly increased in 500 and 1,000 mg/kg males and in females administered 125 mg/kg or greater; sporadic incidences of olfactory epithelium atrophy were observed in females treated with 125, 500, or 1,000 mg/kg (Table 5). Pigment accumulation was characterized by the accumulation of golden brown pigment within macrophages scattered throughout the basal aspect of the olfactory epithelium. The macrophages were more common in areas of olfactory epithelial atrophy but were also present in nonatrophied areas. Minimal pigment was apparent within the cells of the olfactory epithelium. Atrophy was characterized by thinning, decreased cellularity, and disorganization of the olfactory epithelium.

Dose Selection Rationale: The high dose in the 3-month study was based on gavageability and homogeneity of the *Ginkgo biloba* extract dosing solution. The 3-month study did not show any effects on survival or body weights. The hypertrophy observed in histopathologic assessment of the livers and thyroid glands of rats was not considered to be life-threatening. However, the dose spacing was increased from half intervals in the 3-month study to half-log intervals in the 2-year study based on these histopathologic findings in order to capture a range of effect levels. Therefore, the doses selected for the 2-year gavage study in rats were 100, 300, and 1,000 mg/kg.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 3). Survival of 1,000 mg/kg males was significantly less than that of the vehicle controls. However, a notable increase in deaths was not apparent until late in the study beginning around week 85 of exposure. The most frequent cause of early death noted in male rats administered 1,000 mg/kg *Ginkgo biloba* extract was mononuclear cell leukemia. Survival of dosed groups of females was similar to that of the vehicle controls.

TABLE 6
Survival of Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	7	8	10	14
Natural deaths	5	5	9	20
Animals surviving to study termination	38	37	31	16
Percent probability of survival at end of study[a]	76	74	62	32
Mean survival (days)[b]	706	694	695	681
Survival analysis[c]	P<0.001	P=0.993	P=0.190	P<0.001
Female				
Animals initially in study	50	50	50	50
Accidental death[d]	0	0	0	1
Moribund	9	13	7	4
Natural deaths	4	10	6	13
Animals surviving to study termination	37	27	37[e]	32
Percent probability of survival at end of study	74	54	74	66
Mean survival (days)	700	666	702	660
Survival analysis	P=0.897	P=0.051	P=1.000	P=0.402

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal kill).
[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 vehicle controls are in the dosed group columns.
[d] Censored from survival analysis
[e] Includes one animal that died during the last week of the study

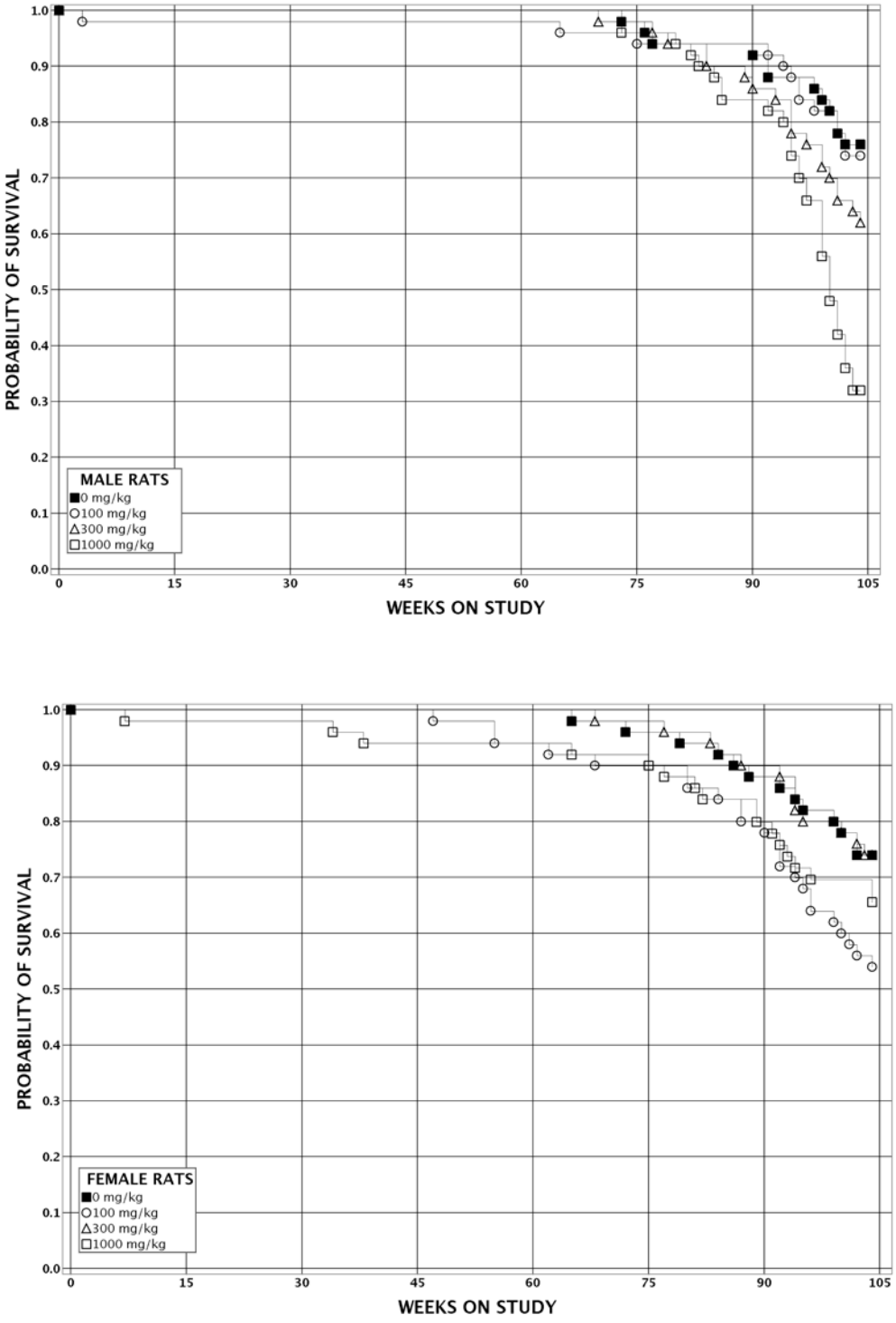


FIGURE 3
Kaplan-Meier Survival Curves for Rats Administered *Ginkgo biloba* Extract by Gavage for 2 Years

Thyroid Hormones

At week 14, all dosed groups of males and the 1,000 mg/kg female group in the special study had increased levels of thyroid stimulating hormone compared to those of the vehicle controls (Tables 7 and F2). The increase appeared dose-related in male rats. There were no statistically significant changes in the levels of triiodothyronine or total thyroxine.

Body Weights and Clinical Findings

Mean body weights of 300 mg/kg males and females were less (10% or more) than those of the vehicle controls after week 93, and those of 1,000 mg/kg males and females were less after week 89 (Figure 4, Tables 8 and 9). Clinical findings included ruffled fur in seven, eight, and 10 males in the 100, 300, and 1,000 mg/kg groups, respectively, beginning at week 89; four vehicle control males also had ruffled fur.

TABLE 7
Thyroid Hormone Data for Special Study Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Male				
n	9	10	10	10
Thyroid stimulating hormone (ng/mL)				
Day 22	8.33 ± 0.47	8.38 ± 0.89[b]	10.78 ± 1.49[c]	11.20 ± 0.96
Week 14	6.89 ± 0.56	9.10 ± 0.50**	9.60 ± 0.75**	10.90 ± 0.81**
Female				
n	9	10	10	10
Thyroid-stimulating hormone (ng/mL)				
Day 22	9.43 ± 0.48[d]	10.00 ± 0.82[c]	9.56 ± 0.53[c]	10.44 ± 0.56[c]
Week 14	5.56 ± 0.34	5.70 ± 0.33	6.40 ± 0.54	7.30 ± 0.40**

** Significantly different (P≤0.01) from the vehicle control group by Shirley's test
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=8
[c] n=9
[d] n=7

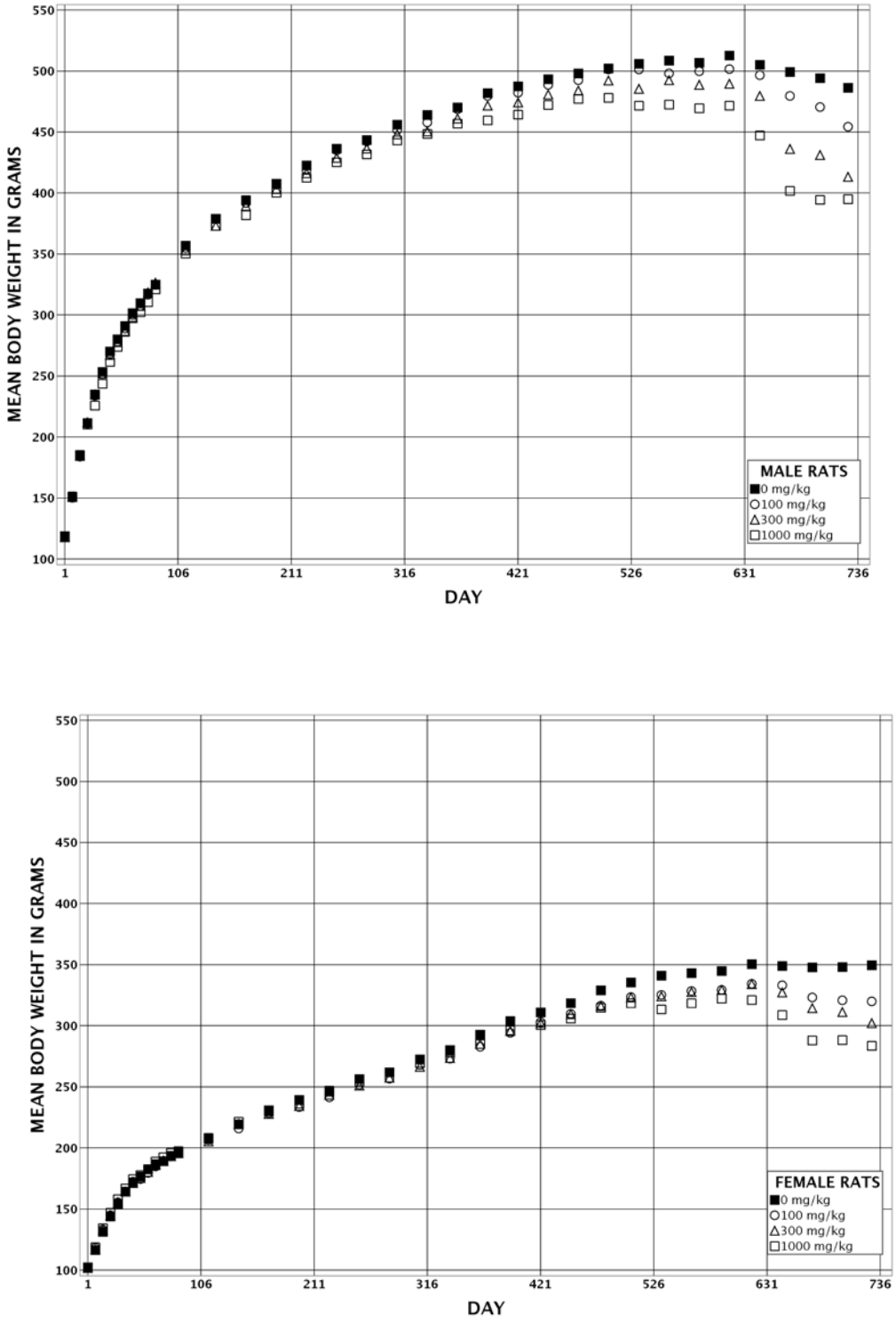


FIGURE 4
Growth Curves for Rats Administered *Ginkgo biloba* Extract by Gavage for 2 Years

TABLE 8
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control		100 mg/kg			300 mg/kg			1,000 mg/kg		
Day	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	119	50	118	100	50	118	99	50	119	100	50
8	151	50	150	100	50	151	100	50	151	100	50
15	185	50	184	100	49	185	100	50	185	100	50
22	212	50	211	100	49	212	100	50	210	99	50
29	235	50	234	100	49	235	100	50	226	96	50
36	254	50	252	99	49	252	99	50	244	96	50
43	270	50	268	99	49	267	99	50	262	97	50
50	280	50	278	99	49	278	99	50	274	98	50
57	291	50	289	99	49	287	99	50	286	98	50
64	302	50	300	100	49	298	99	50	298	99	50
71	310	50	307	99	49	307	99	50	302	98	50
78	318	50	317	100	49	319	100	50	311	98	50
85	325	50	325	100	49	327	100	50	321	99	50
113	357	50	355	100	49	353	99	50	351	98	50
141	379	50	379	100	49	373	99	50	373	99	50
169	394	50	392	99	49	390	99	50	382	97	50
197	408	50	407	100	49	403	99	50	401	98	50
225	423	50	419	99	49	416	98	50	413	98	50
253	436	50	435	100	49	429	98	50	425	98	50
281	444	50	442	100	49	436	98	50	432	97	50
309	456	50	452	99	49	448	98	50	443	97	50
337	464	50	458	99	49	451	97	50	449	97	50
365	470	50	469	100	49	461	98	50	457	97	50
393	482	50	480	100	49	472	98	50	460	95	50
421	488	50	482	99	49	474	97	50	465	95	50
449	493	50	489	99	48	481	97	50	472	96	50
477	498	50	493	99	48	484	97	50	477	96	50
505	502	49	501	100	48	492	98	49	478	95	49
533	506	47	501	99	47	485	96	49	472	93	48
561	509	47	498	98	47	493	97	47	473	93	47
589	507	47	500	99	47	489	96	45	470	93	45
617	513	47	502	98	47	490	96	44	472	92	42
645	505	44	497	98	46	480	95	42	447	89	41
673	499	44	480	96	42	436	87	39	402	81	34
701	494	40	470	95	41	431	87	35	395	80	23
Mean for Weeks											
1-13	250		249	100		249	99		245	98	
14-52	418		415	100		411	98		408	98	
53-101	497		489	98		474	95		457	92	

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control		100 mg/kg			300 mg/kg			1,000 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
1	102	50	102	99	50	102	100	50	102	99	50
8	117	50	118	101	50	117	101	50	119	102	50
15	131	50	133	101	50	134	102	50	134	102	50
22	144	50	145	101	50	145	101	50	147	102	50
29	154	50	156	101	50	155	101	50	158	103	50
36	164	50	164	100	50	164	100	50	167	102	50
43	172	50	173	100	50	171	100	50	175	101	50
50	177	50	175	99	50	176	99	50	178	101	49
57	183	50	179	98	50	180	99	50	183	100	49
64	187	50	185	99	50	186	99	50	189	101	49
71	189	50	189	100	50	189	100	50	192	102	49
78	194	50	193	100	50	193	100	50	196	101	49
85	196	50	196	100	50	196	100	50	198	101	49
113	208	50	206	99	50	205	99	50	208	100	49
141	220	50	216	98	50	220	100	50	221	101	49
169	231	50	229	99	50	228	99	50	230	100	49
197	239	50	234	98	50	234	98	50	236	99	49
225	246	50	242	98	50	244	99	50	247	100	49
253	257	50	252	98	50	251	98	50	255	99	48
281	262	50	257	98	50	258	98	50	262	100	47
309	273	50	268	98	50	266	98	50	270	99	47
337	280	50	273	97	49	274	98	50	278	99	47
365	293	50	283	97	49	285	97	50	285	97	47
393	304	50	294	97	47	296	97	50	296	98	47
421	311	50	303	98	47	303	97	50	301	97	47
449	319	50	310	97	46	310	97	50	306	96	46
477	329	49	316	96	45	316	96	49	315	96	46
505	336	48	324	96	45	323	96	49	319	95	46
533	341	48	325	95	45	324	95	48	314	92	44
561	343	47	329	96	43	328	96	48	319	93	44
589	345	46	329	95	42	329	95	46	322	93	41
617	351	44	334	95	40	334	95	45	321	92	41
645	349	43	333	95	36	327	94	44	309	89	37
673	348	41	323	93	32	314	90	40	288	83	34
701	348	39	321	92	30	311	89	39	288	83	34
Mean for Weeks											
1-13	162		162	100		162	100		164	101	
14-52	246		242	98		242	99		245	100	
53-101	332		317	96		315	95		306	93	

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 liver, thyroid gland, nose, uterus, kidney, parathyroid gland, glandular stomach, bone marrow, and pituitary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, 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.

Liver: At 14 weeks, absolute and relative liver weights of all dosed groups of special study rats were significantly greater than those of the vehicle control groups (Table G2). At 2 years, the incidences of hepatocellular adenoma in the 100 and 300 mg/kg males were both 3/50, which exceeds the historical control range for corn oil gavage studies (0% to 2%), but is within the historical control range for all routes of administration (0% to 6%) (Tables 10, A1, and A2). Hepatocellular adenomas were generally well circumscribed neoplasms composed of well differentiated hepatocytes that were variable in size and tinctorial characteristics; i.e., eosinophilic, basophilic, clear, vacuolated, or an admixture.

The incidences of centrilobular hepatocyte hypertrophy and bile duct hyperplasia in all dosed groups of males and females were significantly greater than those in the vehicle controls, and the severity generally increased with dose (Tables A4 and B4). Hepatocyte hypertrophy was characterized by enlargement of centrilobular hepatocytes with increased amounts of eosinophilic cytoplasm and was often varied in its presence and severity between lobes and within regions of the same lobe. Bile duct hyperplasia was characterized by increased numbers of bile ducts within portal triads, and in more severely affected animals, it extended into the parenchyma.

The incidence of oval cell hyperplasia was significantly increased in 1,000 mg/kg males (Tables 10 and A4). The lesion was characterized by numerous small ovoid cells with indistinct cytoplasm within and adjacent to portal areas or extending into the surrounding hepatic lobules. The cells were often interspersed with foci of moderate to severe bile duct hyperplasia.

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Hepatocyte, Hypertrophy[a]	1 (1.0)[b]	17** (1.4)	26** (2.1)	27** (2.7)
Bile Duct, Hyperplasia	32 (1.1)	43** (1.5)	46** (2.0)	46** (2.0)
Oval Cell, Hyperplasia	0	1 (2.0)	1 (1.0)	10** (1.8)
Necrosis	1 (3.0)	4 (1.5)	6 (2.0)	7* (2.0)
Degeneration, Cystic	4 (1.0)	14** (1.1)	10 (1.0)	14** (1.1)
Hepatocellular Adenoma[c]	0	3	3	0
Female				
Number Examined Microscopically	50	50	50	50
Hepatocyte, Hypertrophy	7 (1.4)	15* (1.7)	27** (2.2)	33** (2.5)
Bile Duct, Hyperplasia	11 (1.0)	31** (1.1)	31** (1.1)	33** (1.1)
Fatty Change, Focal	11	25**	30**	25**

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 3/299 (1.0% ± 1.1%), range 0%-2%; all routes: 18/1,249 (1.4% ± 1.9%), range 0%-6%

The incidence of necrosis was significantly increased in 1,000 mg/kg males (Tables 10 and A4). Necrosis was characterized by focal, widely scattered, randomly distributed areas of necrotic hepatocytes often infiltrated by small numbers of mixed inflammatory cells. Necrosis was not diagnosed when it was deemed to be secondary to mononuclear cell leukemia.

The incidences of cystic degeneration were significantly increased in 100 and 1,000 mg/kg males (Tables 10 and A4). The lesion was characterized by small numbers of microscopic cyst-like spaces present focally within the hepatic parenchyma. These spaces were filled with wispy, pale eosinophilic material or, occasionally, red blood cells.

Significant, but not dose related, increases in the incidences of focal fatty change occurred in all dosed groups of females (Tables 10 and B4). The lesion was characterized by a focal area of hepatocytes that displayed localized

microvesicular and macrovesicular fatty change. The hepatocytes also contained increased amounts of eosinophilic cytoplasm, large open-faced nuclei, and occasionally multiple nucleoli (Plate 1). Numerous microgranulomas were scattered throughout the lesions and were composed predominantly of macrophages with fewer lymphocytes, plasma cells, and occasional neutrophils. The macrophages often contained fine, acicular clefts (cholesterol clefts). Occasional multinucleated giant cells were also present. Microgranulomas were much more numerous in the focal fatty change than in the surrounding hepatic parenchyma, and histiocytes were a much more prominent cellular component of them. Occasional mitotic figures were also present in hepatocytes and portal triads and central veins were preserved within the lesions. The overall size of the lesion was quite variable, ranging from a few to many hepatic lobules. The number of areas displaying focal fatty change tended to increase with dose.

The incidence of basophilic focus was significantly increased in 100 mg/kg males; the incidences of this lesion were decreased in all other dosed groups of males and females, but not significantly (Tables A4 and B4). Basophilic focus was characterized by a focal area in which the hepatocytes were slightly shrunken and had increased cytoplasmic basophilia. The overall size of basophilic foci was highly variable. Portal areas and central veins were typically preserved in both clear cell and basophilic foci.

The incidences of clear cell focus were significantly decreased in 300 and 1,000 mg/kg males and 100 mg/kg females (Tables A4 and B4). Clear cell focus consisted of a focal area in which the hepatocytes had abundant clear areas within their cytoplasm. The clear areas were irregularly shaped and nuclei tended to be centrally located, consistent with glycogen accumulation. Clear cell foci were generally small, typically measuring less than one or two hepatic lobules in diameter. The reduced incidence of the foci may be explained in part (in the males), by the increased incidences of mononuclear cell leukemia seen in 300 and 1,000 mg/kg males, which potentially masked the presence of foci.

Thyroid Gland: The incidences of follicular cell adenoma were increased, though not significantly, in 300 and 1,000 mg/kg males and 300 mg/kg females (Tables 11, A1, A2, B1, and B2). The incidences in these groups exceeded the historical control range for corn oil gavage studies, and the incidences in 1,000 mg/kg males and

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Male				
Number Examined Microscopically	50	50	49	45
Follicular Cell, Hypertrophy[a]	13 (1.0)[b]	37** (1.2)	41** (1.3)	41** (1.8)
Follicle Hyperplasia	0	7** (1.3)	9** (2.3)	5* (2.8)
Follicular Cell, Adenoma[c]				
Overall rate[d]	2/50 (4%)	1/50 (2%)	3/49 (6%)	5/45 (11%)
Adjusted rate[e]	4.3%	2.2%	6.7%	13.2%
Terminal rate[f]	2/38 (5%)	0/37 (0%)	1/31 (3%)	2/16 (13%)
First incidence (days)	727 (T)	685	485	675
Poly-3 test[g]	P=0.040	P=0.503N	P=0.482	P=0.142
Female				
Number Examined Microscopically	49	50	49	49
Follicular Cell, Hypertrophy	15 (1.0)	41** (1.0)	45** (1.1)	48** (2.0)
Follicle Hyperplasia	3 (1.3)	3 (1.0)	1 (2.0)	5 (1.6)
Follicular Cell, Adenoma[h]				
Overall rate	1/49 (2%)	0/50 (0%)	3/49 (6%)	1/49 (2%)
Adjusted rate	2.2%	0.0%	6.6%	2.4%
Terminal rate	1/37 (3%)	0/27 (0%)	3/37 (8%)	0/32 (0%)
First incidence (days)	728 (T)	—[i]	728 (T)	637
Poly-3 test	P=0.573	P=0.519N	P=0.310	P=0.741
Follicular Cell, Carcinoma[j]	0	0	1	1
Follicular Cell, Adenoma or Carcinoma[k]				
Overall rate	1/49 (2%)	0/50 (0%)	4/49 (8%)	2/49 (4%)
Adjusted rate	2.2%	0.0%	8.8%	4.9%
Terminal rate	1/37 (3%)	0/27 (0%)	4/37 (11%)	1/32 (3%)
First incidence (days)	728 (T)	—	728 (T)	637
Poly-3 test	P=0.324	P=0.519N	P=0.183	P=0.469

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 6/299 (2.0% ± 1.3%), range 0%-4%; all routes: 13/1,239 (1.0% ± 1.7%), range 0%-6%
[d] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value 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 Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A lower incidence in a dose group is indicated by **N**.
[h] Historical incidence for corn oil gavage studies: 3/298 (1.0% ± 1.1%), range 0%-2%; all routes: 8/1,186 (0.7% ± 1.0%), range 0%-4%
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence for corn oil gavage studies: 1/298 (0.3% ± 0.8%), range 0%-2%; all routes: 5/1,186 (0.4% ± 1.0%), range 0%-2%
[k] Historical incidence for corn oil gavage studies: 4/298 (1.4% ± 1.0%), range 0%-2%; all routes: 12/1,186 (1.0% ± 1.3%), range 0%-4%

300 mg/kg females exceeded the historical control range for all routes of study (Tables 11, A3a, and B3a). Single incidences of follicular cell carcinoma occurred in the 300 and 1,000 mg/kg female groups. Adenomas tended to be larger with more compression of the adjacent parenchyma and more complex epithelial infoldings than hyperplasia (Plate 2). Follicular cell carcinomas (Plate 3) were typically large lesions that were highly cellular, although small follicles with pale colloid were typically present throughout the neoplasms. Invasion was present in some neoplasms. Mitoses and cellular atypia were present in many carcinomas. The incidences of follicular cell hypertrophy were significantly increased in all dosed groups of males and females (Tables 11, A4, and B4); severity of the lesion increased with increasing dose in both males and females. The incidences of follicle hyperplasia were significantly increased in all dosed groups of males, and severity of the lesion generally increased with increasing dose in males and females. Follicular cell hypertrophy was characterized by enlargement of the follicular epithelial cells. The cells were cuboidal and had finely vacuolated cytoplasm (Plates 4 and 5). Overall, a decreased amount of colloid was present in the affected animals and the colloid had a slightly basophilic hue. Follicle hyperplasia was characterized by enlarged follicles that typically compressed the surrounding parenchyma. The follicles were lined by hyperplastic follicular epithelium and epithelial lined septae, and papillary projections frequently projected into the follicular colloid (Plate 6). Except for the relative weight of 100 mg/kg males, thyroid gland weights were not significantly increased in special study rats at 14 weeks (Table G2).

Nose: Two incidences of adenoma of the respiratory epithelium occurred in 300 mg/kg females and the incidence exceeded the historical control range for all routes of administration (0% to 2%) (Tables 12, B1, and B3b). Respiratory epithelium adenomas were characterized by an exophytic mass, with a pedunculated or sessile base, growing into the lumen of the nasal passage. The mass consisted of papillary and invaginating, gland-like structures that were composed of pseudostratified epithelium on a scant, fibrovascular stroma.

Except for respiratory epithelium hyperplasia in 100 mg/kg females, the incidences of transitional and respiratory epithelium hyperplasia in all dosed groups were significantly greater than those in the vehicle control groups and the severity of these lesions generally increased with dose (Tables 12, A4, and B4). Hyperplasia of the transitional epithelium was most apparent on the lateral aspects of the nasal cavity at Level I. Normal thickness was considered to be two to three cell layers; minimal hyperplasia was four to five cell layers, mild hyperplasia was six to seven cell layers; moderate hyperplasia was eight to nine cell layers, and marked hyperplasia was more than 10 cell layers.

TABLE 12
Incidences of Neoplasms and Nonneoplastic Lesions of the Nose in Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Male				
Number Examined Microscopically	50	49	49	50
Transitional Epithelium, Hyperplasia[a]	2 (1.5)[b]	18** (1.2)	43** (1.9)	31** (2.5)
Respiratory Epithelium, Hyperplasia	14 (1.6)	28** (1.4)	45** (1.8)	35** (2.2)
Olfactory Epithelium, Atrophy	1 (1.0)	26** (1.3)	37** (1.6)	31** (2.2)
Olfactory Epithelium, Respiratory Metaplasia	9 (1.3)	30** (1.5)	40** (2.0)	32** (2.5)
Nerve, Olfactory Epithelium, Atrophy	0	17** (1.4)	14** (2.1)	23** (2.5)
Olfactory Epithelium, Pigmentation	0	39** (1.5)	42** (1.7)	30** (2.1)
Inflammation, Chronic Active	33 (1.2)	32 (1.3)	38 (1.9)	46** (2.2)
Goblet Cell, Respiratory Epithelium, Hyperplasia	20 (1.5)	18 (1.2)	41** (1.7)	34** (2.1)
Submucosa, Fibrosis	0	0	0	8** (1.6)
Olfactory Epithelium, Hyaline Droplet Accumulation	45 (1.9)	43 (1.4)	14** (1.1)	0**
Female				
Number Examined Microscopically	49	49	50	46
Transitional Epithelium, Hyperplasia	0	6** (1.5)	32** (1.8)	36** (2.8)
Respiratory Epithelium, Hyperplasia	9 (1.2)	9 (1.3)	19* (1.7)	34** (2.3)
Olfactory Epithelium, Atrophy	0	18** (1.1)	25** (1.6)	37** (2.1)
Olfactory Epithelium, Respiratory Metaplasia	8 (1.3)	4 (1.3)	32** (2.0)	37** (2.5)
Nerve, Olfactory Epithelium, Atrophy	0	15** (1.1)	22** (1.6)	33** (2.2)
Olfactory Epithelium, Pigmentation	0	37** (1.5)	43** (2.0)	40** (1.9)
Inflammation, Chronic Active	22 (1.0)	16 (1.2)	26 (1.5)	38** (1.9)
Goblet Cell, Respiratory Epithelium, Hyperplasia	6 (1.2)	2 (1.0)	18** (1.6)	35** (1.8)
Olfactory Epithelium, Hyaline Droplet Accumulation	44 (2.0)	39 (1.7)	25** (1.2)	0**
Respiratory Epithelium, Adenoma[c]				
Overall rate[d]	0/49 (0%)	0/49 (0%)	2/50 (4%)	0/46 (0%)
Adjusted rate[e]	0.0%	0.0%	4.4%	0.0%
Terminal rate	0/37 (0%)	0/27 (0%)	2/37 (5%)	0/32 (0%)
First incidence (days)[f]	—[g]	—	728 (T)	—
Poly-3 test[h]	P=0.710N	—[i]	P=0.242	—

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 0/299; all routes: 1/1,196 (0.1% ± 0.4%), range 0%-2%
[d] Number animals with neoplasm per number of animals with nose examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Not applicable; no neoplasms in animal group
[h] Beneath the vehicle control incidence is the P value 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 Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend is indicated by **N**.
[i] Value of the statistic cannot be computed.

Hyperplasia of the respiratory epithelium typically occurred in the ventral aspect of Level III in association with inflammation but also occasionally occurred in other areas of Levels I and II. It was characterized by increased thickness of the respiratory epithelium resulting from an increase in the number of cells. Typically, an increased number of layers of nuclei were present in the hyperplastic areas. In more severe cases, the epithelium had an undulating appearance resulting from the hyperplasia. The severity score was based on both the extent of the hyperplasia throughout the nasal cavity and the degree of hyperplasia in the areas where it was present.

In the olfactory epithelium, the incidences of atrophy, nerve atrophy, and pigmentation were significantly increased in all dosed groups of males and females, the incidences of respiratory metaplasia were increased in all dosed groups of males and in 300 and 1,000 mg/kg females, and the severity of these lesions generally increased with increasing dose (Tables 12, A4, and B4). Atrophy of the olfactory epithelium was characterized by thinning and disorganization of the olfactory epithelial layer with a loss of the epithelial brush border (Plates 7 and 8). The atrophy ranged in severity from minimal to moderate and was localized to nasal Levels II and III. In cases of minimal atrophy, the incidences of respiratory metaplasia were significantly increased in all dosed males and in 300 and 1,000 mg/kg females, and atrophy was typically limited to the peripheral aspects of the nasal cavity, especially the ventral aspect of the nasal septum. With increasing severity, the epithelium was both thinner and the lesion was more widespread throughout the nasal cavity. Respiratory metaplasia of the olfactory epithelium was characterized by complete replacement of the olfactory epithelium by respiratory epithelium. This change was most common in the peripheral (especially ventral) regions of the nasal cavity of Level III but in severe cases involved large areas including the more central areas of the nasal cavity. Atrophy of the nerve in the olfactory epithelium was commonly diagnosed in the animals that also had olfactory epithelial atrophy, which is an expected finding given that the nerves are composed of axons of the olfactory epithelial neurons. The severity scores generally paralleled the olfactory epithelial atrophy severity scores. Pigment accumulation in the olfactory epithelium was principally characterized by the accumulation of macrophages containing golden brown pigment within the basal aspect of the olfactory epithelium and occasionally in the submucosa (Plate 9). The pigment accumulation was largely restricted to the atrophic areas of the olfactory epithelium of Levels II and III, although small amounts were also occasionally located in areas displaying respiratory metaplasia. Pigment was also present within the olfactory epithelial cells but this was also present in many vehicle control animals, although often to a lesser degree. Grading was subjectively

based on the amount of pigment within the macrophages and the number of pigment-containing macrophages throughout the epithelium.

The incidences of chronic active inflammation were significantly increased in 1,000 mg/kg males and females (Tables 12, A4, and B4), and the severity increased with increasing dose. Chronic active inflammation was characterized by a mixed inflammatory infiltrate that involved the submucosa and the lumen of the nasal cavity (Plate 10). The inflammatory infiltrate in the submucosa was composed of a mixture of neutrophils, macrophages, lymphocytes, and plasma cells whereas the inflammatory exudate within the nasal cavity was predominantly suppurative, containing large amounts of neutrophils mixed with necrotic debris. The inflammation in the dosed groups occurred most commonly in the posterior aspects of the nasal cavity and was generally most severe ventrally. It was not uncommon for 25% to 50% of the nasal cavity at Level III to be filled with inflammatory exudates. The inflammation commonly extended into the nasopharyngeal duct. In these animals it was common for the nasopharyngeal ductular epithelium to be hyperplastic and for the submucosa to contain infiltrates of mixed inflammatory cells.

The incidences of hyperplasia of the goblet cells in the respiratory epithelium were significantly increased in 300 and 1,000 mg/kg males and females; the severity was also increased in these groups (Tables 12, A4, and B4). Goblet cell hyperplasia was characterized by increased numbers of goblet cells throughout the respiratory epithelium.

Submucosal fibrosis occurred only in 1,000 mg/kg males and the incidence was significantly increased (Tables 12 and A4).

The incidences of hyaline droplet accumulation in the olfactory epithelium were significantly decreased in 300 and 1,000 mg/kg males and females; the severity was also decreased (Tables 12, A4, and B4). Hyaline droplet accumulation in the olfactory epithelium was often present, particularly in the vehicle control animals. Hyaline droplet accumulation was characterized by the accumulation of hyalinized, eosinophilic material in the cytoplasm of cells of the olfactory epithelium, and much less commonly, the respiratory epithelium. This change was present in

nasal Levels II and III. The amount of the epithelium affected varied considerably between animals. When a grade of minimal was assigned, the accumulation of eosinophilic material was barely detectable in one to a few focal areas of olfactory epithelium in the nasal cavity (less than 10%). A grade of mild was assigned when the material was present in 10% to 25% of the olfactory epithelium; moderate or marked was assigned when the eosinophilic material was present in 25% to 50% or 50% to 75% of the olfactory epithelium, respectively. The treatment-related decrease in the normally present hyaline droplets may reflect discharge of the cytoplasmic contents, protecting from the irritant effect of the test compound or its metabolites.

Mononuclear Cell Leukemia: The incidences of mononuclear cell leukemia in 300 and 1,000 mg/kg males were significantly greater than that in the vehicle controls and exceeded the historical control range for corn oil gavage studies (8%-28%) but not the range for all routes (8%-58%) (Tables 13, A1, A2, and A3b). A comparable range of organs involved by systemic mononuclear cell leukemia was observed in vehicle control and treated males. The splenic parenchyma was typically effaced by large numbers of leukemic cells resulting in a massively enlarged spleen in most cases.

Uterus: The incidence of stromal polyp was significantly increased in 1,000 mg/kg females (0 mg/kg, 3/50; 100 mg/kg, 8/50; 300 mg/kg, 8/50; 1,000 mg/kg, 9/50) and the incidences of cystic endometrial hyperplasia were significantly increased in 100 and 300 mg/kg females (3/50, 13/50, 11/50, 7/50) (Tables B1, B2, and B4). Stromal polyps typically were epithelial lined masses of fibrovascular endometrial stroma that protruded into the uterine lumen. Attachment to the uterine wall was rarely apparent in the section. Cystic endometrial hyperplasia was characterized by thickening of the endometrial wall due to an increase in the glandular structures and was sometimes accompanied by increased cellularity of the stroma. Although the incidence of stromal polyp in 1,000 mg/kg females slightly exceeded the historical control range for corn oil gavage studies (6%-16%), it did not exceed the range for all routes (4%-34%), and the increase was not considered to be related to *Ginkgo biloba* extract administration (Table B3c).

Kidney: Dose-related increased severity of nephropathy was noted in all dosed male groups (1.7, 2.0, 2.4, 2.9). Nephropathy was variable in appearance depending on the severity. Minimally to mildly affected animals had

TABLE 13
Incidences of Mononuclear Cell Leukemia in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
All Organs: Mononuclear Cell Leukemia[a]				
Overall rate[b]	9/50 (18%)	12/50 (24%)	22/50 (44%)	21/50 (42%)
Adjusted rate[c]	19.2%	25.7%	45.7%	46.8%
Terminal rate[d]	6/38 (16%)	7/37 (19%)	9/31 (29%)	7/16 (44%)
First incidence (days)	626	643	485	568
Poly-3 test[e]	P=0.004	P=0.308	P=0.004	P=0.003

[a] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 53/299 (17.7% ± 6.6%), range 8%-28%; all routes: 450/1,249 (36.0% ± 14.4%), range 8%-58%
[b] Number of animals with mononuclear cell leukemia per number necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value 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 Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

multifocal clusters of tubular regeneration and basement membrane thickening often with minimal to mild interstitial fibrosis, chronic inflammation, and tubular proteinosis. With increasing severity, the kidney changes eventually encompassed nearly 100% of the parenchyma, and marked tubular regeneration, glomerular sclerosis, tubular proteinosis, chronic inflammation, and fibrosis were prominent. Males in the 300 and 1,000 mg/kg groups often had moderate to marked nephropathy.

Parathyroid Gland: The incidence of hyperplasia was significantly increased in 1,000 mg/kg males (1/47, 3/48, 2/48, 7/47; Table A4). The lesion was characterized by an increase in the number of parathyroid cells and its occurrence was considered secondary to the kidney nephropathy that occurred in dosed males.

Glandular Stomach: The incidence of mineralization was significantly increased in 1,000 mg/kg males (1/50, 1/50, 1/50, 6/50; Table A4). Mineralization occurred in the middle of the glandular epithelial layer and was composed of numerous, punctate deeply basophilic foci (mineral). Occurrence of the lesion was considered secondary to marked nephropathy.

Bone Marrow: The incidence of hyperplasia was significantly increased in 1,000 mg/kg males (14/40, 15/50, 21/48, 26/50; Table A4). Hyperplasia was characterized by an increase in the number of hematopoietic cells in the bone

marrow and ranged from minimal to marked severity (data not shown). The cellular composition was mixed and, even in severe cases, the regular organization of the hematopoietic cells was retained.

Pituitary Gland (Pars Distalis): The incidences of adenoma occurred with negative trends in males and females and were significantly decreased in the 300 and 1,000 mg/kg groups (males: 0 mg/kg, 38/50; 100 mg/kg, 30/50; 300 mg/kg, 25/50; 1,000 mg/kg, 19/50; females: 34/50, 28/50, 22/50, 16/50; Tables A1, A2, B1, and B2). A dose-related positive trend in the incidences of hyperplasia occurred in females, and the incidence in the 1,000 mg/kg group was significantly increased (11/50, 13/50, 18/50, 23/50; Table B4). Adenoma was characterized by a variably-sized mass that typically expanded and compressed the remaining pituicytes. Cytologically, the neoplasms were most commonly composed of neoplastic chromophobe cells, although a minority of neoplasms was composed of other pituitary cell lineages such as acidophils. Mitoses and cellular atypia were common in adenomas. Foci of hyperplasia in the pars distalis were generally small and nodular and lacked compression of the adjacent pituitary parenchyma. Minimal cellular atypia and only rare mitoses were present.

MICE

3-MONTH STUDY

One female mouse in the 1,000 mg/kg group died of a dosing accident during week 11 (Table 14 and Figure 5). The final mean body weights and body weight gains of 2,000 mg/kg females were significantly less than those of the vehicle control group. Ruffled fur was observed in two 1,000 mg/kg males between weeks 7 and 8 and all 2,000 mg/kg males between weeks 5 and 9.

In 500 mg/kg or greater female mice, there were significant decreases in the total leukocyte counts, with associated decreases in the differential counts. Reticulocyte counts were decreased in 500 mg/kg or greater males and females, and platelet counts were increased in the 500 mg/kg or greater males (Table F3).

TABLE 14
Survival and Body Weights of Mice in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	21.0 ± 0.3	33.6 ± 0.9	12.6 ± 0.8	
125	10/10	21.1 ± 0.3	34.4 ± 0.6	13.4 ± 0.5	103
250	10/10	21.1 ± 0.2	34.3 ± 0.8	13.2 ± 0.8	102
500	10/10	21.3 ± 0.3	34.0 ± 1.0	12.8 ± 0.9	101
1,000	10/10	20.8 ± 0.2	33.2 ± 0.3	12.4 ± 0.3	99
2,000	10/10	20.9 ± 0.2	32.4 ± 0.6	11.5 ± 0.5	97
Female					
0	10/10	17.9 ± 0.4	27.8 ± 0.3	9.9 ± 0.5	
125	10/10	17.8 ± 0.3	27.9 ± 1.1	10.1 ± 0.9	101
250	10/10	17.6 ± 0.2	27.4 ± 0.5	9.8 ± 0.5	99
500	10/10	17.8 ± 0.3	27.3 ± 0.6	9.5 ± 0.5	98
1,000	9/10[c]	17.9 ± 0.3	26.1 ± 0.5	8.3 ± 0.3	94
2,000	10/10	17.7 ± 0.2	25.4 ± 0.2*	7.8 ± 0.2**	92

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Weights and weight changes are given as mean ± standard error.
[b] Number of animals surviving at 14 weeks/number initially in group
[c] Week of accidental death: 11

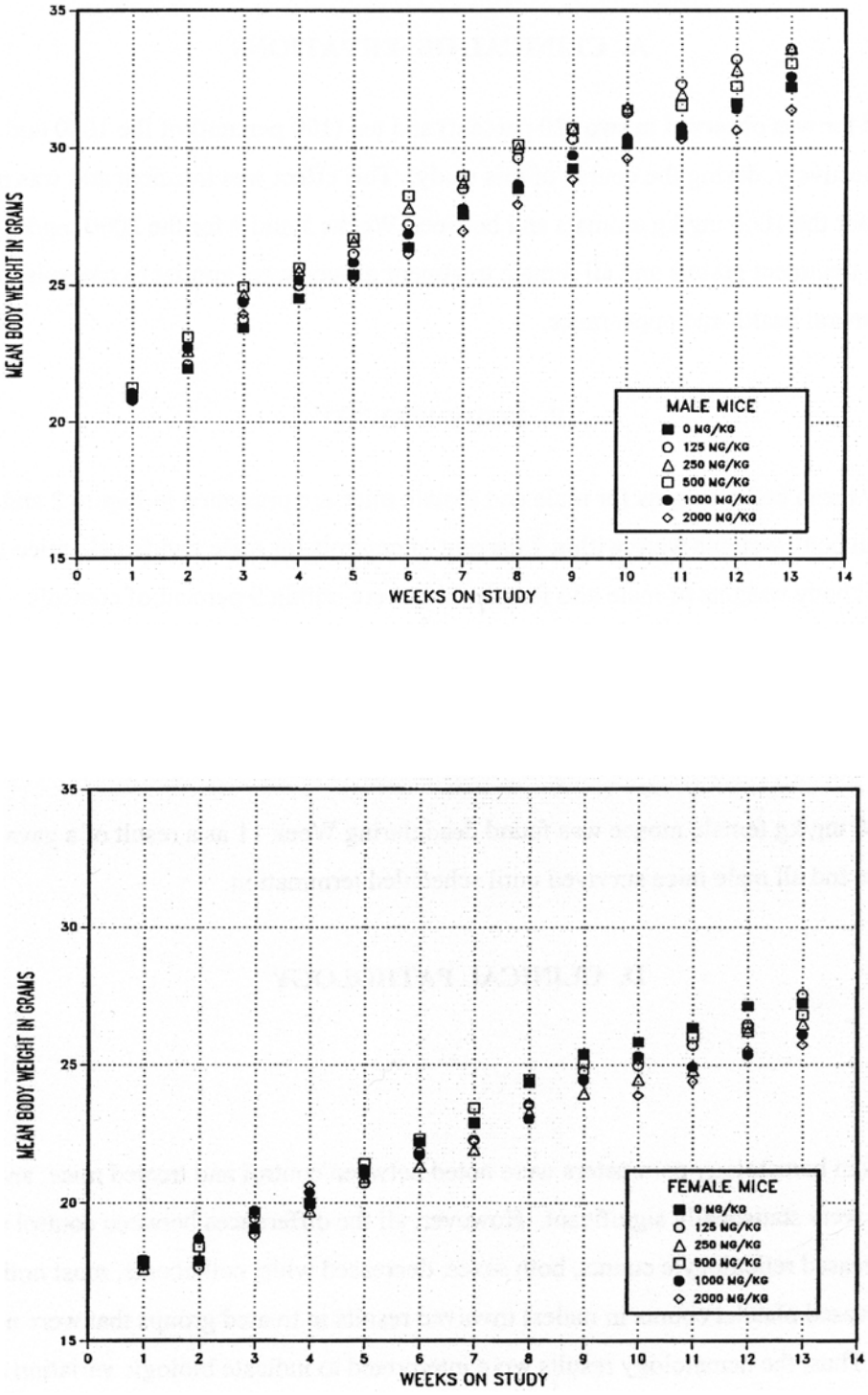


FIGURE 5
Growth Curves for Mice Administered *Ginkgo biloba* Extract by Gavage for 3 Months

Absolute and relative liver weights of 250 mg/kg or greater males and all dosed groups of females were significantly increased relative to those of the vehicle control groups (Table G3). Absolute and relative kidney weights of 2,000 mg/kg males were significantly decreased relative to the vehicle control group.

There were no significant differences in sperm parameters of male mice administered 500, 1,000, or 2,000 mg/kg when compared to the vehicle controls or in the estrous cycles of female mice administered 500 or 1,000 mg/kg (Tables H3 and H4). Female mice administered 2,000 mg/kg had a significantly higher probability of extended estrus when compared to the vehicle controls, which indicates potential for *Ginkgo biloba* extract to be a reproductive toxicant in female mice under these study conditions.

The incidences of hepatocytic hypertrophy were significantly increased in males and females in the 250 mg/kg or greater groups, and the severity of the lesion generally increased with increasing dose in both sexes and was greater in males than females (Table 15). Significantly increased incidences of focal hepatocytic necrosis occurred in 1,000 and 2,000 mg/kg males. Hepatocytic hypertrophy began in the centrilobular areas and expanded with increasing severity to the midzonal areas; the lesion was characterized by an enlargement of the hepatocytes up to approximately one and a half times the normal size. Hypertrophic hepatocytes had increased amounts of cytoplasm and slightly enlarged nuclei. Focal hepatocytic necrosis was a slight change consisting of one, and occasionally more, minute foci of hepatocytes with brightly eosinophilic cytoplasm and pyknotic or fragmented nuclei.

The incidences of hyaline droplet accumulation in the respiratory epithelium of the nose were significantly increased in 500 mg/kg males and 1,000 and 2,000 mg/kg females (Table 15). The incidences of hyaline droplet accumulation in the olfactory epithelium were significantly increased in males in the 500 and 1,000 mg/kg groups and females in the 125, 500, and 1,000 mg/kg groups. The incidences of atrophy of the olfactory epithelium were significantly increased in 1,000 mg/kg males and females. The incidences of pigment accumulation in macrophages in the olfactory epithelium were significantly increased in males in the 500 mg/kg or greater groups and in 1,000 and 2,000 mg/kg females. Hyaline droplet accumulation was characterized by an accumulation of globular hyaline material in the cytoplasm of respiratory or olfactory epithelial cells. The globules occupied sub- or supra-nuclear positions and stained brightly eosinophilic with hematoxylin and eosin. Atrophy of the olfactory epithelium was

TABLE 15
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Male						
Liver[a]	10	10	10	10	10	10
Hepatocyte, Hypertrophy[b]	0	0	10** (1.4)[c]	10** (1.7)	10** (2.7)	10** (2.7)
Hepatocyte, Necrosis, Focal	0	0	1 (1.0)	0	5* (1.0)	9** (1.0)
Nose	10	10	10	10	10	10
Respiratory Epithelium, Accumulation, Hyaline Droplet	1 (1.0)	3 (1.0)	4 (1.0)	9** (1.3)	2 (2.0)	4 (1.0)
Olfactory Epithelium, Accumulation, Hyaline Droplet	1 (1.0)	1 (1.0)	4 (1.3)	9** (1.0)	8** (1.4)	5 (1.0)
Olfactory Epithelium, Hyaline Droplet, Atrophy	0	0	1 (3.0)	2 (1.5)	5* (1.8)	3 (1.7)
Olfactory Epithelium, Pigmentation	0	0	0	7** (1.0)	7** (1.6)	8** (1.0)
Female						
Liver	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	0	4* (1.0)	10** (1.2)	9** (1.6)	10** (1.9)
Nose	10	10	10	10	10	10
Respiratory Epithelium, Accumulation, Hyaline Droplet	2 (1.0)	4 (1.0)	4 (1.0)	6 (1.5)	9** (1.7)	8* (1.1)
Olfactory Epithelium, Accumulation, Hyaline Droplet	1 (1.0)	7** (1.0)	3 (1.0)	6* (1.3)	8** (1.4)	5 (1.0)
Olfactory Epithelium, Hyaline droplet, Atrophy	0	0	0	0	5* (1.2)	1 (2.0)
Olfactory Epithelium, Pigmentation	0	0	0	3 (1.0)	7** (1.4)	6** (1.2)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

characterized by decreased numbers of nuclei (sustentacular and olfactory neurons), mild disorganization of the nuclei, and was often associated with increased numbers of pigment-containing macrophages. Scattered individual or small clusters of apoptotic cells were frequently noted in the areas of atrophy. The olfactory epithelium on the tips of the nasal turbinates and lining the lateral surfaces of the posterior nasal cavity, particularly in the more ventral aspect of the Level III section, was most often affected. Pigment accumulation was characterized by macrophages with an abundant amount of golden-brown granular cytoplasm. Macrophages were usually located immediately above the basal cell layer and below the nuclear layer of the sustentacular cells and olfactory neurons.

Macrophages containing pigment were often noted near areas of atrophy of the olfactory epithelium, but the presence of the macrophages themselves did not appear to cause the atrophy.

Dose Selection Rationale: The high dose in the 3-month study was based on gavageability and homogeneity limitations of the *Ginkgo biloba* extract dosing solution. *Ginkgo biloba* administration in the 3-month study did not have any effects on survival. Body and organ weight changes and liver hypertrophy observed in the histopathologic assessment were not considered to be life-threatening. However, the dose spacing was increased from half intervals in the 3-month study to half-log intervals in the 2-year study based on these histopathologic findings in order to capture a range of effect levels. Therefore, the doses selected for the 2-year gavage study in mice were 200, 600, and 2,000 mg/kg.

2-YEAR STUDY

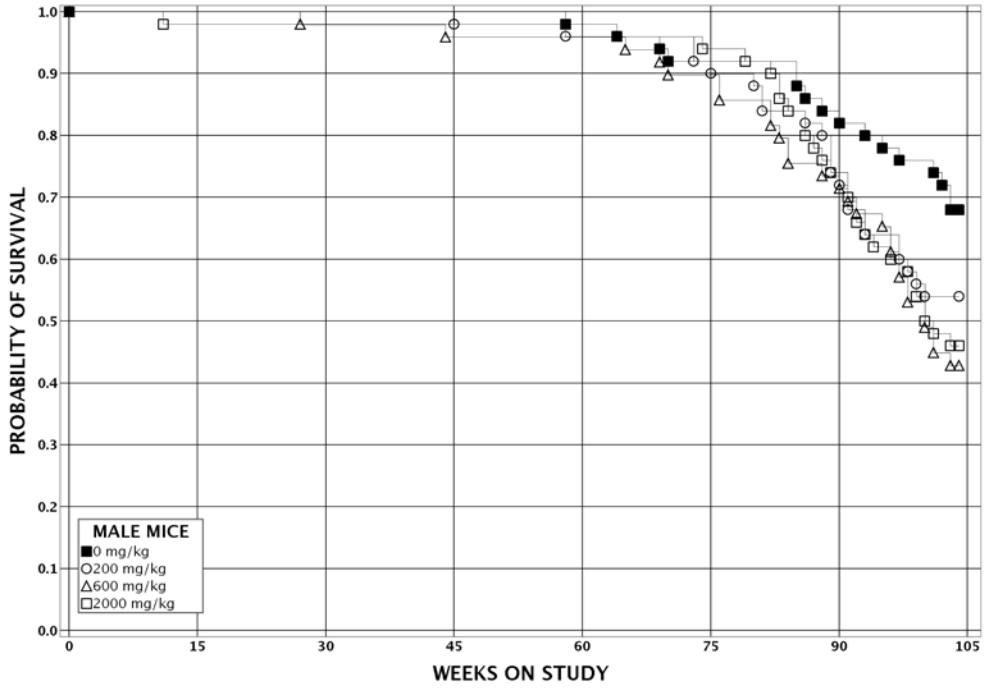
Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 16 and in the Kaplan-Meier survival curves (Figure 6). Survival of 600 and 2,000 mg/kg males was significantly less than that of the vehicle controls. The majority of early deaths in the 600 and 2,000 mg/kg males was due to liver tumors. Survival of 600 mg/kg females was significantly greater than that of the vehicle controls.

TABLE 16
Survival of Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	0	1	0
Moribund	8	16	23	13
Natural deaths	8	7	5	14
Animals surviving to study termination	34	27	21	23
Percent probability of survival at end of study[b]	68	54	43	46
Mean survival (days)[c]	688	665	637	661
Survival analysis[d]	P=0.129	P=0.180	P=0.017	P=0.039
Female				
Animals initially in study	50	50	50	50
Accidental deaths[a]	5	2	1	0
Moribund	8	8	3	3
Natural deaths	6	4	3	11
Animals surviving to study termination	31	36	43	36
Percent probability of survival at end of study	69	76	88	72
Mean survival (days)	629	700	698	701
Survival analysis	P=0.947	P=0.648N	P=0.048N	P=0.938N

[a] Censored from survival analysis
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal kill).
[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 vehicle controls are in the dosed group columns. A lower mortality in a dosed group is indicated by **N**.



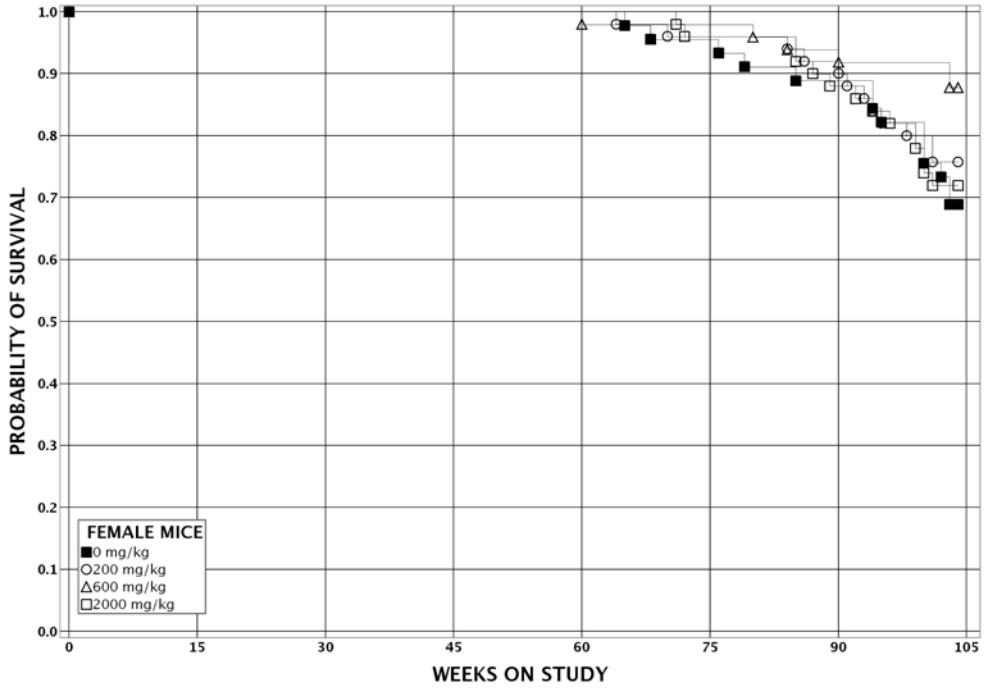


FIGURE 6
Kaplan-Meier Survival Curves for Mice Administered *Ginkgo biloba* Extract by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of 600 and 2,000 mg/kg males were less (10% or more) than those of the vehicle controls after weeks 85 and 77, respectively; mean body weights of 2,000 mg/kg females were generally less than those of the vehicle controls between weeks 17 and 69 and after week 93 (Figure 7; Tables 17 and 18). There were no clinical findings related to *Ginkgo biloba* extract administration.

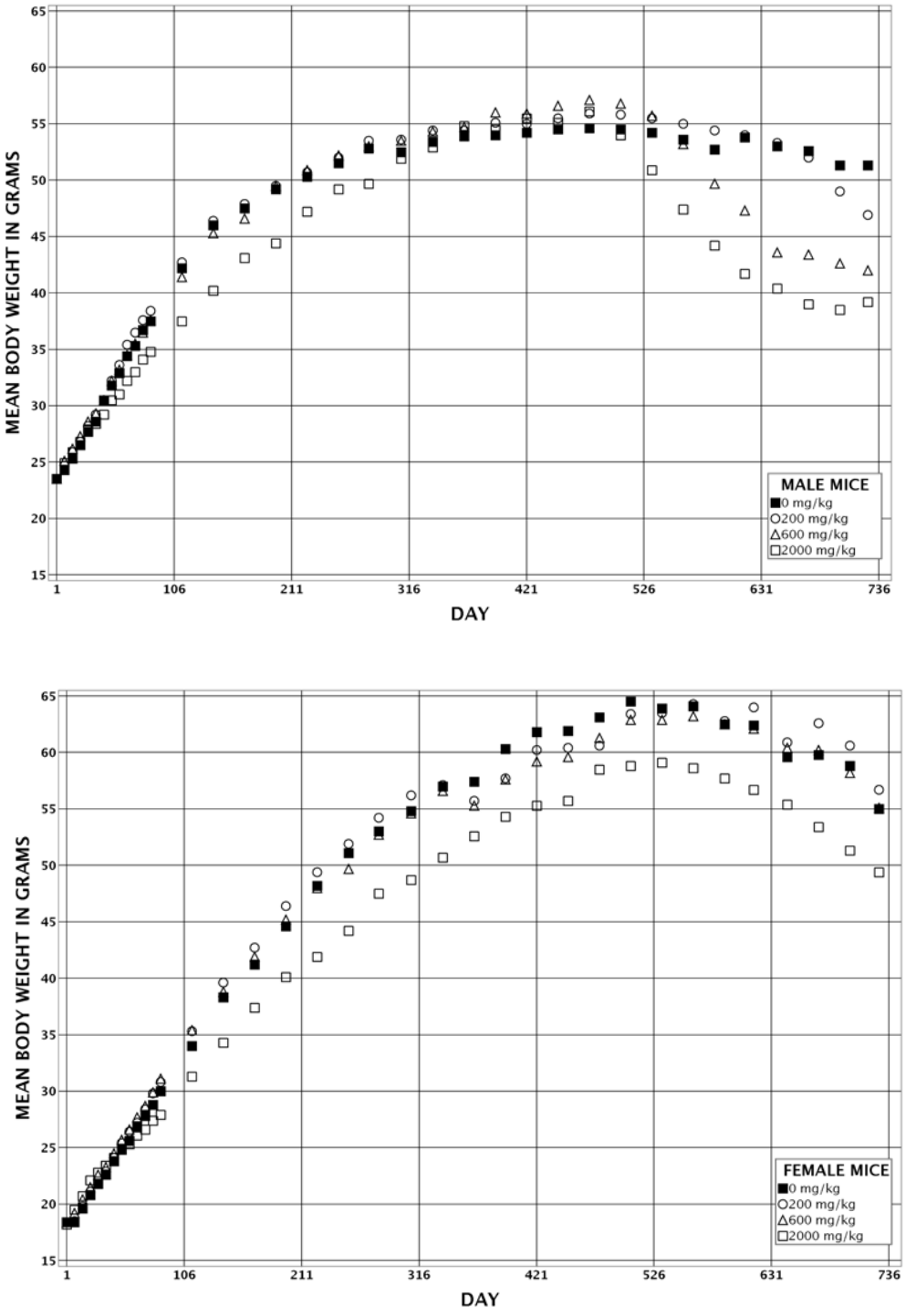


FIGURE 7
Growth Curves for Mice Administered *Ginkgo biloba* Extract by Gavage for 2 Years

TABLE 17
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control		200 mg/kg			600 mg/kg			2,000 mg/kg		
Day	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	23.5	100	50	23.5	100	50	23.5	100	50
8	24.3	50	24.7	102	50	25.1	103	49	24.9	103	50
15	25.3	50	25.9	102	50	26.2	104	49	25.9	103	50
22	26.5	50	26.8	101	50	27.3	103	49	26.8	101	50
29	27.7	50	28.2	102	50	28.6	103	49	27.9	101	50
36	28.6	50	29.2	102	50	29.3	103	49	28.4	99	50
43	30.5	50	30.5	100	50	30.5	100	49	29.2	96	50
50	31.8	50	32.2	101	50	32.2	101	49	30.5	96	50
57	32.9	50	33.6	102	50	33.2	101	49	31.0	94	50
64	34.4	50	35.4	103	50	34.5	100	49	32.2	93	50
71	35.3	50	36.5	103	50	35.5	101	49	33.0	94	50
78	36.7	50	37.6	103	50	36.5	100	49	34.1	93	49
85	37.5	50	38.4	102	50	37.6	100	49	34.8	93	49
113	42.2	50	42.7	101	50	41.4	98	49	37.5	89	49
141	46.0	50	46.4	101	50	45.3	99	49	40.2	87	49
169	47.5	50	47.9	101	50	46.6	98	49	43.1	91	49
197	49.2	50	49.5	101	50	49.5	101	48	44.4	90	49
225	50.3	50	50.7	101	50	50.9	101	48	47.2	94	49
253	51.5	50	52.0	101	50	52.2	101	48	49.2	96	49
280	52.8	50	53.5	101	50	53.0	100	48	49.7	94	49
309	52.5	50	53.6	102	50	53.5	102	47	51.9	99	49
337	53.4	50	54.4	102	49	54.3	102	47	52.9	99	49
365	53.9	50	54.4	101	49	54.7	101	47	54.8	102	49
393	54.0	50	55.1	102	49	56.0	104	47	54.5	101	49
421	54.2	49	55.0	101	48	55.9	103	47	55.5	102	49
449	54.5	48	55.5	102	48	56.6	104	47	55.1	101	48
477	54.6	48	55.9	102	48	57.1	105	45	56.1	103	48
505	54.5	46	55.8	102	47	56.8	104	44	54.0	99	48
533	54.2	46	55.5	102	45	55.7	103	42	50.9	94	47
561	53.6	46	55.0	103	44	53.2	99	42	47.4	88	46
589	52.7	46	54.4	103	42	49.7	94	37	44.2	84	42
616	53.8	42	54.0	100	40	47.3	88	36	41.7	78	38
645	53.0	41	53.3	101	32	43.6	82	33	40.4	76	33
673	52.6	39	52.0	99	32	43.4	82	29	39.0	74	30
701	51.3	38	49.0	96	27	42.6	83	23	38.5	75	25
Mean for Weeks											
1-13	30.4		31.0	102		30.8	101		29.4	97	
14-52	49.5		50.1	101		49.6	100		46.2	93	
53-101	53.6		54.2	101		51.7	96		48.6	91	

TABLE 18
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control		200 mg/kg			600 mg/kg			2,000 mg/kg		
Day	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	18.4	50	18.4	100	50	18.4	100	50	18.2	99	50
8	18.4	47	18.5	101	50	19.2	104	49	19.5	106	50
15	19.6	45	19.8	101	50	20.4	104	49	20.7	106	50
22	20.8	45	21.1	101	50	21.5	103	49	22.1	106	50
29	21.8	45	21.8	100	50	22.6	104	49	22.8	105	50
36	22.6	45	22.9	101	50	23.3	103	49	23.4	103	50
43	23.8	45	24.1	101	50	24.5	103	49	24.1	102	50
50	24.8	45	25.4	102	50	25.7	104	49	24.9	100	50
57	25.6	45	26.4	103	50	26.6	104	49	25.3	99	50
64	26.8	45	27.2	102	50	27.7	104	49	26.1	98	50
71	27.8	45	28.4	102	50	28.7	103	49	26.6	96	50
78	28.8	45	29.8	103	50	29.9	104	49	27.4	95	50
85	30.0	45	30.8	103	50	31.1	104	49	27.9	93	50
113	34.0	45	35.3	104	50	35.4	104	49	31.3	92	50
141	38.3	45	39.6	104	50	38.8	101	49	34.3	90	50
169	41.2	45	42.7	104	50	41.9	102	49	37.4	91	50
197	44.6	45	46.4	104	50	45.2	101	49	40.1	90	50
225	48.2	45	49.4	103	50	48.0	100	49	41.9	87	50
253	51.1	45	51.9	102	50	49.7	97	49	44.2	87	50
280	53.0	45	54.2	102	50	52.7	100	49	47.5	90	50
309	54.8	45	56.2	103	50	54.6	100	49	48.7	89	50
337	57.0	45	57.1	100	50	56.6	99	49	50.7	89	50
365	57.4	45	55.7	97	50	55.3	96	49	52.6	92	50
393	60.3	45	57.7	96	50	57.6	96	49	54.3	90	50
421	61.8	45	60.2	98	50	59.2	96	48	55.3	90	50
449	61.9	45	60.4	98	49	59.6	96	48	55.7	90	50
477	63.1	43	60.6	96	49	61.3	97	48	58.5	93	50
505	64.5	43	63.4	98	48	62.9	98	48	58.8	91	48
533	63.9	42	63.5	100	48	62.9	99	48	59.1	93	48
561	64.1	41	64.3	100	48	63.2	99	47	58.6	91	48
589	62.5	40	62.8	101	47	62.5	100	46	57.7	92	47
615	62.4	40	64.0	103	46	62.1	99	46	56.7	91	45
645	59.6	40	60.9	102	44	60.4	101	45	55.4	93	43
673	59.8	37	62.6	105	41	60.2	101	45	53.4	89	41
701	58.8	34	60.6	103	38	58.2	99	45	51.3	87	37
Mean for Weeks											
1-13	23.8		24.2	102		24.6	103		23.8	101	
14-52	46.9		48.1	103		47.0	100		41.8	89	
53-101	61.5		61.3	100		60.4	98		56.0	91	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant lymphoma and neoplasms and/or nonneoplastic lesions of the liver, thyroid gland, forestomach, nose, and lung. Summaries of the incidences of neoplasms and nonneoplastic lesions, 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.

A number of gross lesions were noted in vehicle control and dosed mice at necropsy. These included masses and nodules in the liver, nodules and foci in the forestomach, and enlargement of the Harderian gland and spleen. Microscopic lesions were found that correlated with these gross findings and often indicated a chemical-related effect.

Liver: The incidences of multiple hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma were increased in all dosed groups of males; multiple hepatocellular adenoma incidences were increased in all dosed groups of females, and multiple hepatocellular carcinoma and hepatoblastoma incidences were increased in 600 and 2,000 mg/kg females (Tables 19, C1, and D1). When single and multiple neoplasm incidences were combined, significant increases were seen in the incidences of hepatocellular adenoma in 200 mg/kg males and all dosed groups of females, hepatocellular carcinoma in all dosed groups of males and 2,000 mg/kg females, and hepatoblastoma in all dosed groups of males and 600 and 2,000 mg/kg females (Tables 19, C2, and D2). These significantly increased incidences also exceeded the historical control ranges for these neoplasms from corn oil gavage studies and all routes of administration (except for hepatocellular adenoma in 200 mg/kg females) (Tables 19, C3a, and D3).

The incidences of hepatocellular adenoma or hepatocellular carcinoma (combined) were significantly increased in all dosed groups of males and females; incidences of these combined lesions exceeded the historical control ranges for corn oil gavage studies and all routes of administration in males, and the historical control ranges for corn oil gavage studies in females (Tables 19, C1, C2, C3a, D1, D2, and D3). The incidences of hepatocellular carcinoma or hepatoblastoma (combined) were significantly increased in all dosed groups of males and in 2,000 mg/kg females;

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Hypertrophy[a]	3 (1.7)[b]	19** (2.6)	35** (3.0)	23** (3.2)
Erythrophagocytosis	0	4* (2.0)	11** (1.2)	7** (1.3)
Hematopoietic Cell Proliferation	4 (1.0)	9 (1.1)	12* (1.2)	14** (1.0)
Inflammation	28 (1.2)	35 (1.5)	42** (1.8)	39** (1.8)
Necrosis	9 (1.9)	15 (2.1)	17* (1.9)	19* (2.3)
Basophilic Focus	15	14	5*	4**
Clear Cell Focus	21	22	14	12*
Mixed Cell Focus	15	13	12	9
Hepatocellular Adenoma, Multiple	18	40**	26**	27**
Hepatocellular Adenoma (includes multiple)[c]				
Overall rate[d]	31/50 (62%)	46/50 (92%)	33/50 (66%)	33/50 (66%)
Adjusted rate[e]	66.2%	96.0%	74.3%	71.0%
Terminal rate[f]	22/34 (65%)	27/27 (100%)	16/21 (76%)	16/23 (70%)
First incidence (days)	482	400	308	443
Poly-3 test[g]	P=0.171N	P<0.001	P=0.260	P=0.388
Hepatocellular Carcinoma, Multiple	11	23**	32**	43**
Hepatocellular Carcinoma (includes multiple)[h]				
Overall rate	22/50 (44%)	31/50 (62%)	41/50 (82%)	47/50 (94%)
Adjusted rate	47.0%	70.6%	92.5%	98.8%
Terminal rate	13/34 (38%)	20/27 (74%)	20/21 (95%)	23/23 (100%)
First incidence (days)	442	555	490	553
Poly-3 test	P<0.001	P<0.015	P<0.001	P<0.001
Hepatocellular Adenoma or Carcinoma[i]				
Overall rate	39/50 (78%)	46/50 (92%)	46/50 (92%)	49/50 (98%)
Adjusted rate	79.4%	96.0%	98.5%	100.0%
Terminal rate	25/34 (74%)	27/27 (100%)	21/21 (100%)	23/23 (100%)
First incidence (days)	442	400	308	443
Poly-3 test	P<0.001	P=0.011	P=0.002	P<0.001
Hepatoblastoma, Multiple	1	12**	26**	21**
Hepatoblastoma (includes multiple)[j]				
Overall rate	3/50 (6%)	28/50 (56%)	36/50 (72%)	38/50 (76%)
Adjusted rate	6.8%	61.0%	80.6%	79.6%
Terminal rate	3/34 (9%)	13/27 (48%)	14/21 (67%)	16/23 (70%)
First incidence (days)	726 (T)	400	477	516
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma or Hepatoblastoma[k]				
Overall rate	24/50 (48%)	42/50 (84%)	45/50 (90%)	48/50 (96%)
Adjusted rate	51.3%	88.3%	97.1%	99.5%
Terminal rate	15/34 (44%)	23/27 (85%)	20/21 (95%)	23/23 (100%)
First incidence (days)	442	400	477	516
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[l]				
Overall rate	39/50 (78%)	48/50 (96%)	48/50 (96%)	49/50 (98%)
Adjusted rate	79.4%	98.5%	100.0%	100.0%
Terminal rate	25/34 (74%)	27/27 (100%)	21/21 (100%)	23/23 (100%)
First incidence (days)	442	400	308	443
Poly-3 test	P<0.001	P=0.002	P<0.001	P<0.001

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Male (continued)				
Number Examined Microscopically	50	50	50	50
Hepatocholangiocarcinoma[m]	0	2	0	1
Female				
Number Examined Microscopically	50	50	50	50
Hypertrophy	0	18** (2.2)	37** (2.1)	37** (2.9)
Erythrophagocytosis	0	3 (1.0)	7* (1.0)	16** (1.0)
Hematopoietic Cell Proliferation	14 (1.5)	12 (1.3)	9 (1.3)	4** (1.8)
Inflammation	38 (1.3)	45 (1.6)	46 (1.3)	41 (1.5)
Vacuolization Cytoplasmic	18 (1.7)	38** (2.1)	44** (2.6)	35** (2.3)
Necrosis	4 (2.3)	2 (2.0)	6 (1.5)	11 (2.0)
Eosinophilic Focus	26	39*	43**	45**
Mixed Cell Focus	7	27**	31**	31**
Hepatocellular Adenoma, Multiple	10	30**	37**	43**
Hepatocellular Adenoma (includes multiple)[n]				
Overall rate	17/50 (34%)	37/50 (74%)	41/50 (82%)	48/50 (96%)
Adjusted rate	41.0%	78.3%	86.9%	96.0%
Terminal rate	14/31 (45%)	29/36 (81%)	38/43 (88%)	34/36 (94%)
First incidence (days)	589	596	628	497
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma, Multiple	2	2	5	31**
Hepatocellular Carcinoma (includes multiple)[o]				
Overall rate	9/50 (18%)	10/50 (20%)	15/50 (30%)	44/50 (88%)
Adjusted rate	21.7%	21.8%	32.1%	91.7%
Terminal rate	5/31 (16%)	8/36 (22%)	14/43 (33%)	34/36 (94%)
First incidence (days)	653	656	718	593
Poly-3 test	P<0.001	P=0.596	P=0.197	P<0.001
Hepatocellular Adenoma or Carcinoma[p]				
Overall rate	20/50 (40%)	39/50 (78%)	41/50 (82%)	49/50 (98%)
Adjusted rate	47.7%	82.1%	86.9%	98.0%
Terminal rate	15/31 (48%)	30/36 (83%)	38/43 (88%)	35/36 (97%)
First incidence (days)	589	596	628	497
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatobalstoma, Multiple	0	0	1	4
Hepatoblastoma (includes multiple)[q]				
Overall rate	1/50 (2%)	1/50 (2%)	8/50 (16%)	11/50 (22%)
Adjusted rate	2.5%	2.2%	17.1%	23.8%
Terminal rate	1/31 (3%)	1/36 (3%)	8/43 (19%)	8/36 (22%)
First incidence (days)	727 (T)	727 (T)	727 (T)	609
Poly-3 test	P<0.001	P=0.735N	P=0.028	P=0.004
Hepatocellular Carcinoma or Hepatoblastoma[r]				
Overall rate	10/50 (20%)	11/50 (22%)	18/50 (36%)	44/50 (88%)
Adjusted rate	24.1%	24.0%	38.5%	91.7%
Terminal rate	6/31 (19%)	9/36 (25%)	17/43 (40%)	34/36 (94%)
First incidence (days)	653	656	718	593
Poly-3 test	P<0.001	P=0.594N	P=0.110	P<0.001

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Female (continued)				
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[s]				
Overall rate	20/50 (40%)	39/50 (78%)	41/50 (82%)	49/50 (98%)
Adjusted rate	47.7%	82.1%	86.9%	98.0%
Terminal rate	15/31 (48%)	30/36 (83%)	38/43 (88%)	35/36 (97%)
First incidence (days)	589	596	628	497
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 181/350 (51.7% ± 6.9%), range 44%-62%; all routes: 658/1,149 (57.3% ± 12.6%), range 24%-78%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value 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 Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N.**
[h] Historical incidence for corn oil gavage studies: 116/350 (33.1% ± 10.5%), range 16%-44%; all routes: 399/1,149 (34.7% ± 10.8%), range 16%-56%
[i] Historical incidence for corn oil gavage studies: 239/350 (68.3% ± 8.9%), range 56%-78%; all routes: 844/1,149 (73.5% ± 11.3%), range 52%-90%
[j] Historical incidence for corn oil gavage studies: 14/350 (4.0% ± 2.8%), range 2%-8%; all routes: 61/1,149 (5.3% ± 7.1%), range 0%-34%
[k] Historical incidence for corn oil gavage studies: 125/350 (35.7% ± 9.5%), range 22%-48%; all routes: 433/1,149 (37.7% ± 11.1%), range 18%-58%
[l] Historical incidence for corn oil gavage studies: 242/350 (69.1% ± 8.0%), range 58%-78%; all routes: 852/1,149 (74.2% ± 11.5%), range 52%-92%
[m] Historical incidence for corn oil gavage studies: 7/350 (2.0% ± 3.1%), range 0%-8%; all routes: 13/1,149 (1.1% ± 2.1%), range 0%-8%
[n] Historical incidence for corn oil gavage studies: 75/347 (21.6% ± 10.8%), range 6%-34%; all routes: 380/1,195 (31.8% ± 21.4%), range 2%-78%
[o] Historical incidence for corn oil gavage studies: 29/347 (8.3% ± 5.5%), range 2%-18%; all routes: 144/1,195 (12.1% ± 10.8%), range 0%-46%
[p] Historical incidence for corn oil gavage studies: 91/347 (26.2% ± 12.7%), range 8%-40%; all routes: 444/1,195 (37.2% ± 22.9%), range 6%-82%
[q] Historical incidence for corn oil gavage studies: 1/347 (0.3% ± 0.8%), range 0%-2%; all routes: 4/1,195 (0.3% ± 0.8%), range 0%-2%
[r] Historical incidence for corn oil gavage studies: 30/347 (8.6% ± 6.1%), range 2%-20%; all routes: 148/1,195 (12.4% ± 11.2%), range 0%-46%
[s] Historical incidence for corn oil gavage studies: 91/347 (26.2% ±12.7%), range 8%-40%; all routes: 444/1,195 (37.2% ± 22.9%), range 6%-82%

incidences of these combined lesions in these groups exceeded the historical control ranges for corn oil gavage studies and for all routes of administration. The incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) were significantly increased in all dosed groups of males and females; incidences of these combined lesions exceeded the historical control ranges for corn oil gavage studies and for all routes of administration (all dosed groups except 200 and 600 mg/kg females).

Hepatocellular adenomas were generally well circumscribed neoplasms composed of well-differentiated hepatocytes that were variable in size and tinctorial characteristics (i.e., eosinophilic, basophilic, clear, vacuolated, or an admixture) (Plate 11). Vacuolated cytoplasm was likely due to fatty change. Adenomas had a lack of normal architecture as opposed to foci of alteration, which retained normal architecture. Hepatocytes showed an altered growth pattern and frequently grew at right angles to the cords of hepatocytes at the periphery of the mass. Although compression was present in some adenomas, it was not always a consistent feature. Adenomas varied from solid to growth patterns containing plates of cells one to three cell layers thick. Some adenomas were atypical and consisted of large cells with abundant eosinophilic cytoplasm, large nuclei and areas with numerous mitotic figures (two to three per high-power field). Adenomas frequently had large venous profiles throughout the mass. Fatty change (lipidosis) and eosinophilic intracytoplasmic inclusions were noted in some adenomas. Hepatocellular carcinomas were not always well-demarcated and had irregular borders as cells infiltrated into the surrounding parenchyma. Hepatocellular carcinomas tended to occur as larger masses than adenomas, often replacing nearly all of a lobe of the liver. Cellular atypia and mitotic figures were common. Nucleoli were often enlarged and multiple. Cells had variable tinctorial appearance from eosinophilic, basophilic, vacuolated, or a mixture. Some carcinomas had a solid growth pattern while a trabecular pattern composed of cords of hepatocytes three or more cell layers thick was also common (Plate 12). Trabeculae were separated by dilated vascular spaces. When the solid growth pattern was present, the cells tended to be anaplastic consisting of large cells with large hyperchromatic irregular shaped nuclei, double nuclei, two or three nucleoli, abundant eosinophilic cytoplasm, or scant basophilic cytoplasm, and numerous areas with two or three mitotic figures per high-power field. Hepatoblastomas were characterized by an irregular mass of compacted basophilic neoplastic cells arranged in sheets with palisading around vascular spaces. Nuclei were generally irregularly oval to round with a scant amount of basophilic cytoplasm; mitotic figures were numerous (Plate 13). Blood-filled cystic spaces, necrosis, and hemorrhage were often common.

Hepatoblastomas frequently arose within hepatocellular carcinomas and hepatocellular adenomas. Hepatoblastomas arising within or at the margin of either a hepatocellular adenoma or a hepatocellular carcinoma were recorded as a hepatoblastoma.

The incidences of hemangiosarcoma were significantly decreased in the 600 mg/kg males; single incidences of the lesion occurred in 200 and 600 mg/kg females (Tables C1, C2, and D1). However, the incidence in vehicle control males in the current study is the highest in any gavage study in the historical control database (0%-18%). Hemangiosarcomas in the liver were poorly demarcated vascular neoplasms characterized by neoplastic spindle-shaped endothelial cells lining cords of hepatocytes and producing small to large dilated blood-filled spaces. Nuclei were hyperchromatic, oval to elongate, and often bulged into vascular lumens. Necrosis and hemorrhage were common.

Two incidences of hepatocholangiocarcinoma (single or multiple) occurred in 200 mg/kg males and a single incidence occurred in 2,000 mg/kg males; these incidences were within the historical control ranges for corn oil gavage (0% to 8%) studies and for all routes of administration (0% to 8%) (Tables 19 and C1). Hepatocholangiocarcinomas were characterized by both neoplastic hepatocytes and neoplastic bile ducts. In many areas, the neoplasms were consistent with hepatocellular carcinomas while in smaller areas the neoplasms were typical of cholangiocarcinomas. The hepatocellular component formed sheets and trabeculae while the biliary component formed irregular acinar structures. In some areas there was a transition from hepatocellular carcinoma to cholangiocarcinoma.

The incidences of hepatocytic hypertrophy were significantly increased in all dosed groups of males and females and a dose-related increase in severity occurred in the males (Tables 19, C4, and D4). Hypertrophy was variable in distribution and severity among animals. In males, the hypertrophy first appeared in the midzonal areas and tended to spread to the portal triad and centrilobular areas with increasing severity; in females, the hypertrophy first was noted in the centrilobular areas and then progressed to involve the midzonal and portal areas. Hypertrophy was graded as follows: minimal=less than 20% of hepatocytes affected; mild=20% to 60% of hepatocytes affected; moderate=60% to 80% of hepatocytes affected; marked=greater than 80% of hepatocytes affected.

The incidences of hepatocytic erythrophagocytosis were significantly increased in all dosed groups of males and in the 600 and 2,000 mg/kg females (Tables 19, C4, and D4). Erythrophagocytosis was characterized by enlarged hepatocytes in which the cytoplasm was filled with red blood cells (Plate 14). The nucleus was either marginated to the side of the cell or centrally located surrounded by intracytoplasmic red blood cells. Remaining cytoplasm often formed a distinct band at the peripheral circumference of the hepatocyte.

The incidences of hematopoietic cell proliferation were significantly increased in 600 and 2,000 mg/kg males and significantly decreased in 2,000 mg/kg females (Tables 19, C4, and D4). Hematopoietic cell proliferation was generally characterized by small clusters of nucleated red blood cells and lesser numbers of immature hematopoietic precursor cells in sinusoids, portal areas, or near central veins. In some animals, myeloid precursors were the predominant cell type.

The incidences of inflammation were significantly increased in 600 and 2,000 mg/kg males (Tables 19, C4, and D4). Inflammation in the liver was characterized by foci of mixed inflammatory cells consisting primarily of mononuclear cells and neutrophils in the parenchyma but also including a mixture of lymphocytes, plasma cells, and macrophages in portal areas. Inflammation was recorded when one or more foci of inflammation were noted in nonneoplastic tissue within a section of liver. Inflammation was graded as follows: minimal=1 to 10 foci observed in a typical section; mild=11 to 20 foci observed in a typical section; moderate=21 to 30 foci observed in a typical section; marked=more than 30 foci observed in a typical section.

The incidences of cytoplasmic vacuolization were significantly increased in all dosed groups of females (Tables 19 and D4). Cytoplasmic vacuolization was diagnosed when affected hepatocytes were distended with colorless to lightly eosinophilic, delicately reticulated cytoplasm as seen when hepatocytes contain glycogen. More severely affected livers had discrete round cytoplasmic vacuoles suggestive of lipid as well. Liver vacuolization was graded for severity based on the percentage of vacuolated hepatocytes: minimal=5% to 10%; mild=11% to 20%; moderate=21% to 50%; marked=51% or greater.

Incidences of necrosis were significantly increased in 600 and 2,000 mg/kg males (Tables 19 and C4). Necrosis was diagnosed when one or more focal areas of necrosis (usually coagulative) were randomly distributed within liver sections; necrosis present in neoplasms or within one millimeter of a malignant neoplasm was not recorded. Single-cell necrosis in areas of hypertrophy was not recorded.

The incidences of basophilic focus in 600 and 2,000 mg/kg males and clear cell focus in 2,000 mg/kg males were significantly decreased (Tables 19 and C4). Basophilic foci were randomly distributed in liver sections. Foci were generally oval to round with irregular, although distinct, margins, and hepatic cords were arranged in a relatively normal pattern that merged with the surrounding hepatic cords. Hepatocytes in basophilic foci were generally smaller than normal hepatocytes and had a basophilic cytoplasm (due to the presence of free ribosomes or rough endoplasmic reticulum). Clear cell foci were often small with fairly distinct margins, although cords merged with surrounding normal hepatic cords. Clear cell foci were composed of hepatocytes in which the cytoplasm was less dense or clear due to loss of glycogen during processing. The clear cytoplasm was usually more prominent in the perinuclear region and lacked discrete vacuoles. The hepatocytes were generally of normal size or slightly enlarged and had a centrally located nucleus.

The incidences of eosinophilic foci were significantly increased in all dosed groups of females (Tables 19 and D4). Eosinophilic foci were well circumscribed lesions one to several lobules in diameter and consisted of enlarged hepatocytes with distinct granular eosinophilic cytoplasm. Minimal to slight compression of surrounding hepatocytes was noted. The architecture of the liver lobule was retained, as is generally typical of foci of cellular alteration.

The incidences of mixed cell foci were significantly increased in all dosed groups of females (Tables 19 and D4). Mixed cell foci were generally round to oval and varied from less than one hepatic lobule to up to several lobules in diameter. The hepatic plates merged imperceptibly with the surrounding hepatocytes and caused little to no compression of the surrounding parenchyma. The normal architecture was retained with triad areas and central veins found within the focus. Mixed cell foci were composed of a mixture of hepatocyte cell types as found in

basophilic, eosinophilic, or clear cell foci with no one cell type predominant. Vacuolated hepatocytes containing clear vacuoles with smooth distinct borders consistent with lipid were also common in mixed cell foci.

Incidences of tension lipidosis were significantly decreased in the 600 mg/kg males (Table C4). Tension lipidosis consisted of focal fairly well demarcated areas of hepatocytes containing numerous small, round, well circumscribed vacuoles in the cytoplasm consistent with lipid accumulation, and was noted near the attachment of the gallbladder.

Thyroid Gland: Two incidences each of follicular cell adenoma occurred in the 600 and 2,000 mg/kg male groups (Tables 20 and C1). The incidences in these groups exceeded the historical control ranges for corn oil gavage studies and for all routes of administration (Tables 20 and C3b). Thyroid gland follicular cell adenomas were well circumscribed nonencapsulated masses formed by the proliferation of follicular cells within the thyroid gland (Plate 15). Follicular cell adenomas were small and generally there was minimal compression of surrounding follicles. The neoplastic cells were generally hyperchromatic and formed colloid-containing follicles of variable size. Neoplastic cells formed papillary projections with complex branching protruding into lumens of larger cystically dilated follicles. The neoplastic cells varied from cuboidal to columnar in shape and there was often an increase in the nuclear to cytoplasmic ratio.

The incidence of follicle hyperplasia was significantly increased in 2,000 mg/kg males (Tables 20 and C4). Four 600 mg/kg females also had this lesion (Table D4). Follicle hyperplasia was characterized by follicles lined by a crowded cuboidal to low columnar single cell layer epithelium (Plate 16). Occasional follicles were enlarged with increased colloid while other hyperplastic follicles were small with decreased colloid. In some follicles the epithelium was forming papillary proliferations which protruded into the follicle lumen. Hyperplasia was usually focal to multifocal and only rarely involved the majority of follicles. In no cases were all of the follicles in a gland hyperplastic (diffuse). Foci of hyperplasia were not encapsulated and there was no to minimal compression of the adjacent normal thyroid follicles. Atypia and mitotic figures were not noted.

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Male				
Number Examined Microscopically	49	49	50	50
Follicle, Hyperplasia[a]	2 (1.0)[b]	1 (1.0)	7 (1.1)	25** (1.4)
Follicular Cell Hypertrophy	2 (1.0)	0	2 (1.5)	38** (1.2)
Follicular Cell Adenoma[c]				
Overall rate[d]	0/49 (0%)	0/49 (0%)	2/50 (4%)	2/50 (4%)
Adjusted rate[e]	0.0%	0.0%	5.3%	5.0%
Terminal rate[f]	0/33 (0%)	0/26 (0%)	2/21 (10%)	2/23 (9%)
First incidence (days)	—[g]		726 (T)	726 (T)
Poly-3 test[h]	P=0.125	—[i]	P=0.211	P=0.222
Female				
Number Examined Microscopically	49	48	49	48
Follicular Cell Hypertrophy	1 (3.0)	5 (1.4)	9* (1.0)	39** (1.0)

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 1/349 (0.3% ± 0.8%), range 0%-2%; all routes: 7/1,143 (0.6% ± 1.0%), range 0%-2%
[d] Number animals with neoplasm per number of animals with thyroid gland examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Not applicable; no neoplasms in animal group
[h] Beneath the vehicle control incidence is the P value 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 Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[i] Value of the statistic cannot be computed.

The incidences of follicular cell hypertrophy were significantly increased in 2,000 mg/kg males and 600 and 2,000 mg/kg females (Tables 20, C4, and D4). Follicular cell hypertrophy was characterized by follicles lined by tall cuboidal epithelial cells that were one to two times larger than normal follicle epithelial cells (Plate 17). These cells often contained yellow to pink globules in the cytoplasm or fine vacuoles. Additional features noted in thyroid follicles in which the follicle cells were hypertrophied were abnormal colloid characterized by eosinophilic to basophilic globules, increased sloughed thyroid epithelial cells in the colloid, and frequently enlarged follicles at the periphery of the thyroid gland, especially near the parathyroid glands. Atypia and mitotic figures were not features of hypertrophy.

Forestomach: Squamous cell papilloma (including multiple) occurred in all dosed groups of males (Tables 21 and C1); the incidences were within the historical control range for corn oil gavage studies (0% to16%). Squamous papillomas were characterized by a solitary stalk of lamina propria protruding into the lumen with multiple finger-like projections arising from the stalk. The epithelium covering the projections was usually markedly hyperplastic and covered with excessive keratin; squamous pearl formation was not noted and maturation was fairly orderly. Invasion of epithelium into the stalk or into the submucosa, as seen in squamous cell carcinomas, was not a feature of papillomas.

The incidences of inflammation, epithelium hyperplasia, and epithelium hyperkeratosis were significantly increased in all dosed groups of males and in the 2,000 mg/kg females; the incidences of epithelium ulcer were significantly increased in 2,000 mg/kg males and females (Tables 21, C4, and D4). Epithelium erosion occurred sporadically in all dosed groups of males and in the 200 mg/kg females.

Inflammation was characterized by focal to multifocal infiltrates of a mixed inflammatory infiltrate in the lamina propria in the areas of hyperplasia. Inflammatory infiltrates consisted of lymphocytes, plasma cells, macrophages, neutrophils and eosinophils. Perivascular cuffs of lymphocytes and plasma cells were noted in the submucosa. Neutrophils often migrated into the stratum corneum of the hyperplastic areas to form intracorneal pustules. Epithelium hyperplasia was characterized by focal to multifocal thickening of the squamous epithelium of the forestomach. Multiple, finger-like projections, each with its own lamina propria, oriented perpendicularly to the basal lamina often occurred focally (Plate 18). Maturation was generally orderly and invasion beneath the basement membrane was not noted. Hyperplastic areas of the mucosa were also hyperkeratotic and had inflammation in the lamina propria and often erosions or ulcers in the hyperplastic epithelium. Hyperkeratosis was characterized by increased thickness of the stratum corneum with excessive keratin on the surface of the squamous mucosa. Generally, the hyperkeratosis was orthokeratotic. Hyperkeratosis was noted only in the areas of hyperplasia and the severity of the hyperkeratosis increased with the severity of the hyperplasia. Ulceration and erosion were only noted in the areas of hyperplasia and were characterized by loss of keratinocytes from the squamous epithelium of the

short finger-like projections of hyperplastic squamous epithelium. Erosions were diagnosed when squamous epithelial cells were lost from the hyperplastic epithelium but at least one layer of basal epithelial cells was retained.

TABLE 21
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control		200 mg/kg		600 mg/kg		2,000 mg/kg	
Male								
Forestomach[a]	50		50		50		50	
Inflammation[b]	11	(2.3)[c]	24**	(2.3)	21**	(2.8)	45**	(2.6)
Epithelium, Hyperplasia	14	(2.9)	27**	(3.0)	27**	(3.2)	45**	(3.6)
Epithelium, Hyperkeratosis	11	(2.5)	24**	(3.0)	24**	(3.1)	46**	(3.1)
Epithelium, Ulcer	7	(2.3)	10	(2.5)	12	(2.1)	24**	(2.2)
Epithelium, Erosion	0		2	(2.0)	1	(4.0)	3	(3.3)
Squamous Cell Papilloma[d] (includes multiple)	0		2	(4%)	1	(2%)	1	(2%)
Nose	50		50		50		50	
Olfactory Epithelium Accumulation, Hyaline Droplet	18	(1.4)	16	(1.9)	15	(1.8)	28*	(1.8)
Olfactory Epithelium Pigmentation	0		1	(1.0)	3	(1.0)	13**	(1.1)
Female								
Forestomach	50		50		50		50	
Inflammation	4	(1.8)	6	(2.3)	5	(1.8)	19**	(2.6)
Epithelium, Hyperplasia	8	(2.1)	18*	(1.8)	11	(2.0)	20**	(3.4)
Epithelium, Hyperkeratosis	3	(2.7)	11	(2.0)	5	(1.8)	20**	(2.9)
Epithelium, Ulcer	1	(2.0)	1	(3.0)	1	(2.0)	11*	(2.5)
Epithelium, Erosion	0		1	(3.0)	0		0	
Nose	50		50		50		50	
Olfactory Epithelium Accumulation, Hyaline Droplet	5	(1.0)	3	(1.7)	12	(1.2)	17**	(1.6)
Olfactory Epithelium, Pigmentation	0		1	(1.0)	6*	(1.5)	13**	(1.2)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 10/350 (2.9% ± 5.9%), range 0%-16%; all routes: 22/1,150 (1.9% ± 3.6%), range 0%-16%

Ulcers were diagnosed when epithelial cell loss extended full thickness through the hyperplastic epithelium down to the underlying propria.

Nose: The incidences of hyaline droplet accumulation in the olfactory epithelium were significantly increased in 2,000 mg/kg males and females (Tables 21, C4, and D4). The incidences of pigmentation in the olfactory epithelium were significantly increased in 2,000 mg/kg males and 600 and 2,000 mg/kg females. Hyaline droplet accumulation was characterized by an accumulation of globular hyaline material in the cytoplasm of olfactory epithelial cells. The globules occupied sub- or supranuclear positions and stained brightly eosinophilic with hematoxylin and eosin. The presence of globules was associated with decreased numbers of nuclei, presumably both the sustentacular cells and olfactory neurons. Pigmentation was characterized by macrophages with an abundant amount of golden brown granular cytoplasm (Plate 19). Macrophages were usually located immediately above the basal cell layer and below the nuclear layer of the sustentacular cells and olfactory neurons. Occasionally the macrophages were located in the lamina propria between Bowman's glands. Occasionally a slight increase in numbers of apoptotic nuclei in the nuclear layer of the sustentacular cells and olfactory neurons was noted.

Lung: The incidences of alveolar/bronchiolar adenoma (0 mg/kg, 8/50; 200 mg/kg, 6/50; 600 mg/kg, 5/50; 2,000 mg/kg, 1/50), alveolar/bronchiolar carcinoma (single or multiple combined) (11/50, 8/50, 5/50, 3/50), or alveolar bronchiolar adenoma or carcinoma (combined) (17/50, 14/50, 10/50, 4/50) were significantly decreased in 2,000 mg/kg males (Tables C1 and C2). The combined incidence in this group was also less than the historical control range for all routes (14% to 40%; Table C3c). The incidences of alveolar epithelium hyperplasia were decreased, but not significantly, in 600 and 2,000 mg/kg males (4/50, 3/50, 1/50, 1/50; Table C4).

Malignant Lymphoma: The incidence of malignant lymphoma was significantly decreased in 2,000 mg/kg females (11/50, 9/50, 7/50, 2/50; Tables D1 and D2). Malignant lymphoma was generally either the follicle center lymphoma (mixed cell type) or small cell type. In the follicle center lymphoma, neoplastic cells consisted of a mixture of large and small lymphocytes. The larger cells tended to have a vesicular nucleus with prominent nucleoli and abundant cytoplasm. Mitotic figures were numerous. Small cell lymphoma was characterized by a neoplastic proliferation of a monomorphic population of small to medium sized well differentiated lymphocytes; the mitotic index was low. Malignant lymphoma was generally a systemic process occurring in multiple organs, in addition to the liver.

GENETIC TOXICOLOGY

Ginkgo biloba extract (1,000 to 10,000 µg/plate) was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 and in *Escherichia coli* strain WP2 *uvrA*/pKM101, with and without 10% induced rat liver S9 mix (Table E1). No increase in the frequency of micronucleated erythrocytes was observed in peripheral blood of male B6C3F1/N mice administered *Ginkgo biloba* extract (125 to 2,000 mg/kg per day) for 3 months by gavage (Table E2). In female mice administered these doses, the results of the micronucleus test were judged to be equivocal based on a significant trend test and no individual dose groups being significantly elevated over the vehicle control group (Table E2). No significant alterations in the percentages of reticulocytes (polychromatic erythrocytes) were seen in female mice, suggesting that exposure to *Ginkgo biloba* extract did not cause bone marrow toxicity in the females; in contrast, a significant dose-related decrease in the percentage of circulating reticulocytes was observed in male mice, suggesting that in males, increasing doses of *Ginkgo biloba* extract induced bone marrow toxicity (Table E2).

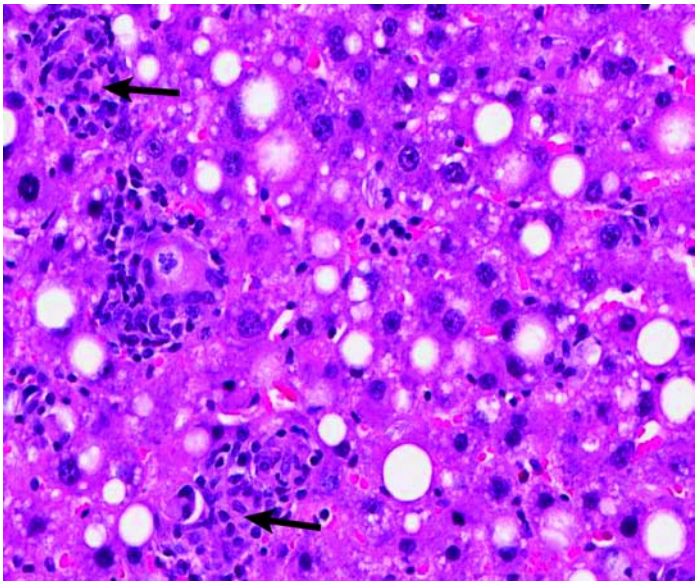


PLATE 1
Focal fatty change in the liver of a female F344/N rat administered 1,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Note hepatocytes displaying microvesicular and macrovesicular fatty change associated with microgranulomas scattered throughout the lesion and composed predominantly of macrophages with fewer lymphocytes, plasma cells, and occasional neutrophils (arrows). The macrophages often contained fine, acicular clefts (cholesterol clefts). H&E

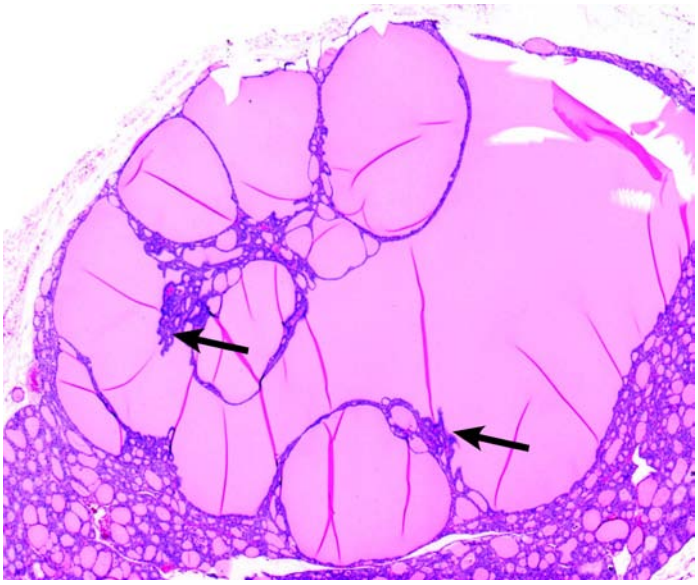


PLATE 2
Thyroid gland follicular cell adenoma in a male F344/N rat administered 1,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. The adenoma tends to be larger with more compression of the adjacent parenchyma and more complex epithelial infoldings than in hyperplasia (arrows). H&E

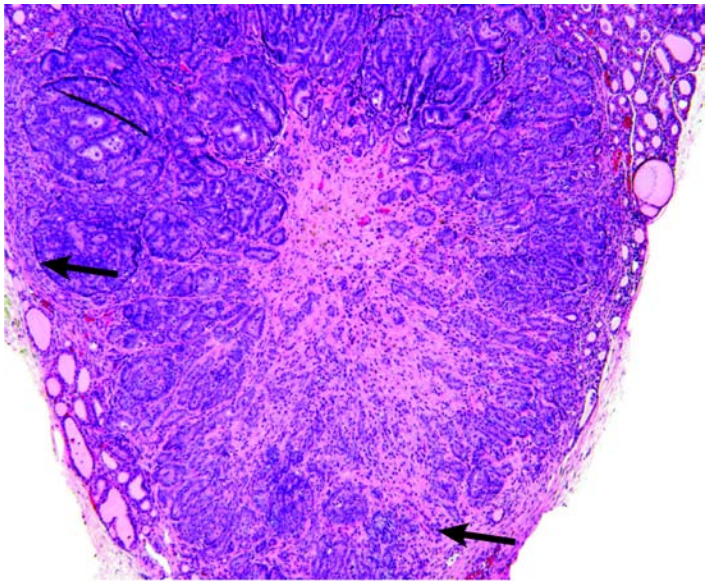


PLATE 3
Thyroid gland follicular cell carcinoma in a female F344/N rat administered 300 mg/kg *Ginkgo biloba* extract by gavage for 2 years. The neoplasm is highly cellular with local invasion (arrows). H&E

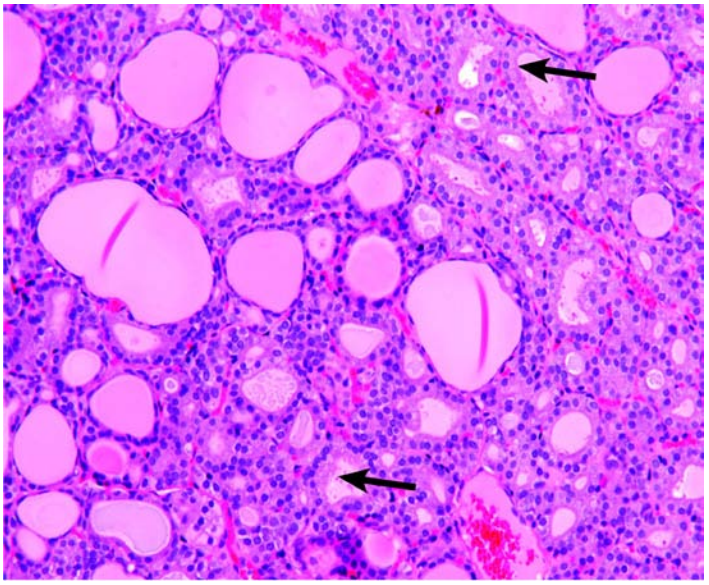


PLATE 4
Thyroid gland follicular cell hypertrophy in a female F344/N rat administered 1,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Most of the follicles are lined by cuboidal epithelium (arrows), and there is a decreased amount of colloid. Compare to Plate 5. H&E

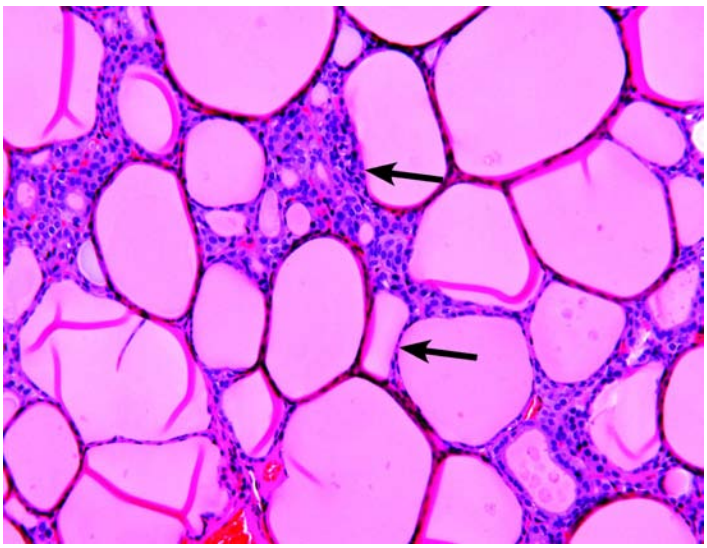


PLATE 5
Normal aspect of thyroid gland follicles from a male vehicle control F344/N rat in the 2-year gavage study of *Ginkgo biloba* extract. Most of the follicles are lined by flattened epithelium (arrows) and the follicles are distended with homogeneous colloid. H&E

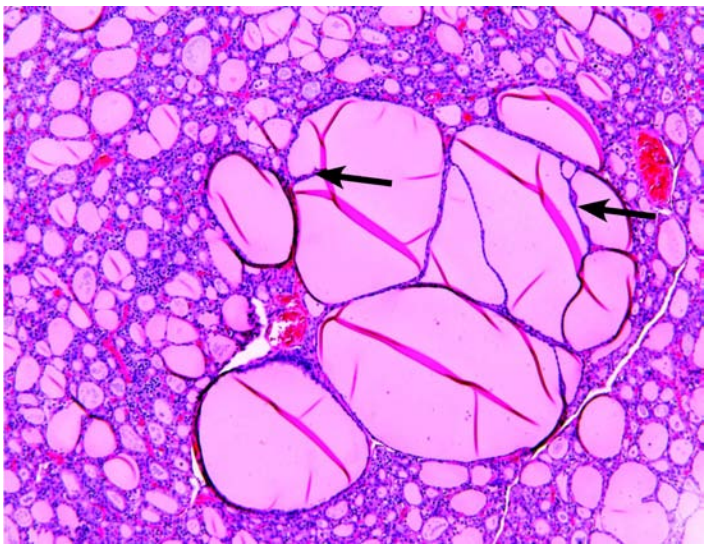


PLATE 6
Thyroid gland follicular cell hyperplasia in a male F344/N rat administered 1,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Note the focal enlargement of follicles that typically compress the surrounding parenchyma. The follicles are lined by hyperplastic follicular epithelium and epithelial-lined septae, and papillary projections (arrows) frequently project into the follicular colloid. H&E

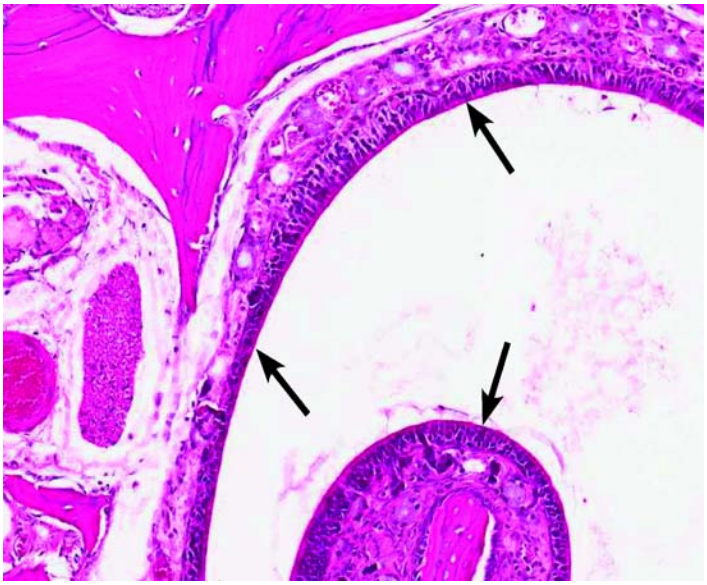


PLATE 7
Atrophy of the olfactory epithelium in the nose (Level III) of a female F344/N rat administered 1,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Note the thinning and disorganization of the olfactory epithelial layer (arrows). Compare to Plate 8. H&E

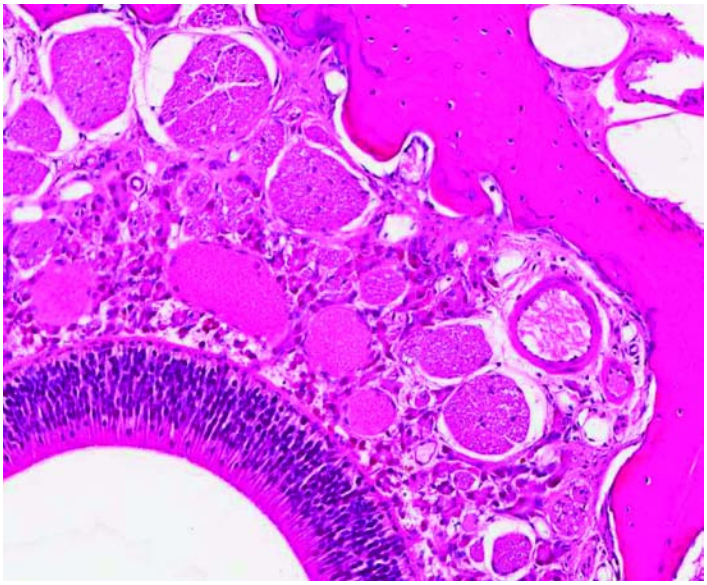


PLATE 8
Normal aspect of the nose (olfactory epithelium, Level III) of a female vehicle control F344/N rat in the 2-year gavage study of *Ginkgo biloba* extract. Note the normal thickness of the epithelium. H&E

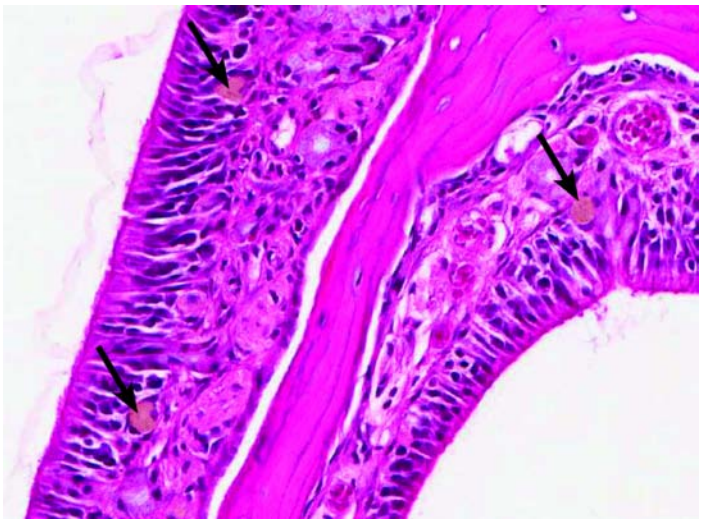


PLATE 9
Atrophy of the olfactory epithelium associated with pigment accumulation in the nose (Level III) of a female F344/N rat administered 1,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Note the thinning of the olfactory epithelial layer, associated with accumulation of macrophages containing golden brown pigment within the basal aspect of the olfactory epithelium and occasionally in the submucosa (arrows). H&E

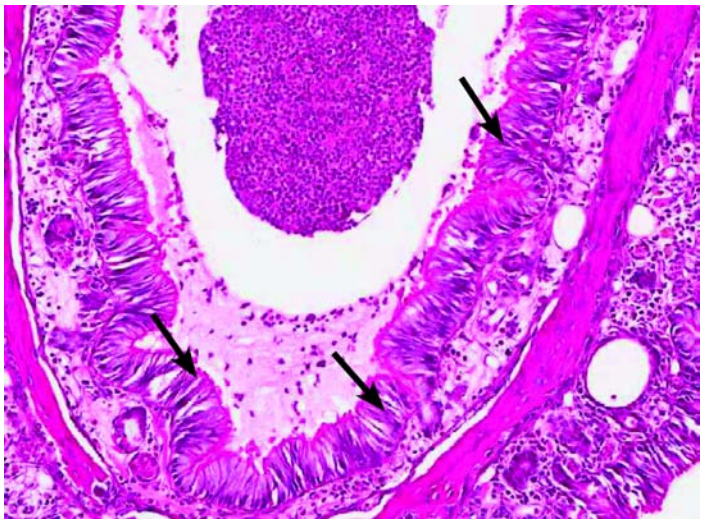


PLATE 10
Chronic active inflammation in the nose (Level III) of a female F344/N rat administered 1,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Note the presence of mixed inflammatory infiltrate that involves the submucosa and the lumen of the nasal cavity. There is also respiratory metaplasia of the olfactory epithelium (arrows). H&E

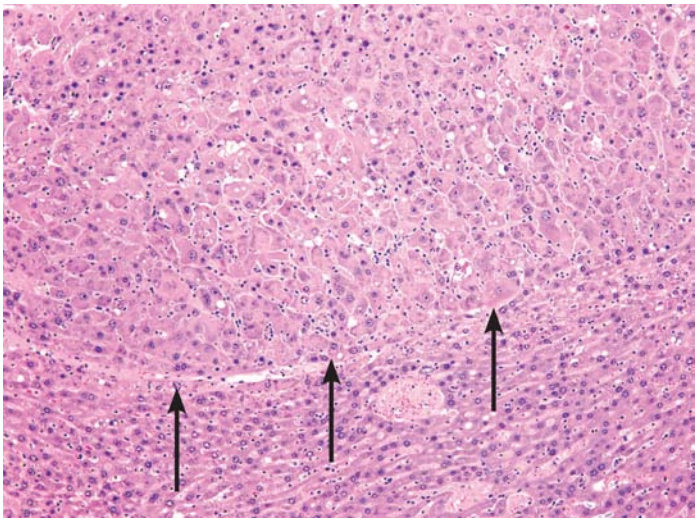


PLATE 11
Hepatocellular adenoma in the liver of a male B6C3F1/N mouse administered 2,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Note the well circumscribed nodule (arrows) composed of well differentiated hepatocytes, having solid growth pattern; the hepatocytes are large cells with abundant eosinophilic cytoplasm. H&E

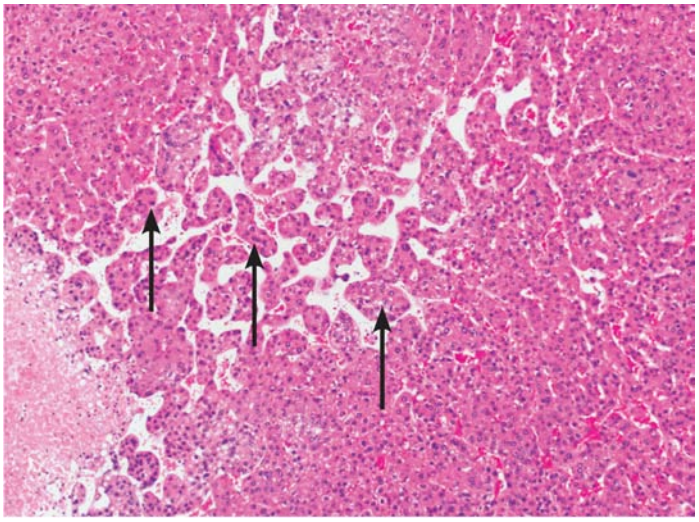


PLATE 12
Hepatocellular carcinoma in the liver of a female B6C3F1/N mouse administered 2,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. The carcinoma has areas with a solid growth pattern and a trabecular pattern composed of cords of hepatocytes three or more cell layers thick (arrows) separated by dilated vascular spaces. H&E

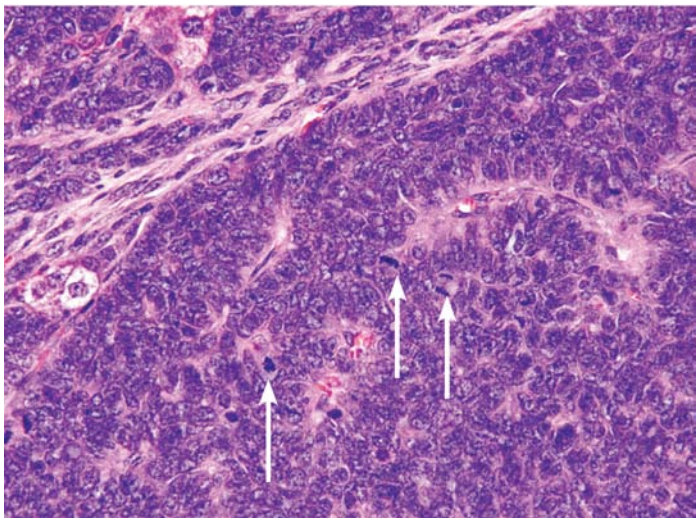


PLATE 13
Hepatoblastoma in the liver of a male B6C3F1/N mouse administered 200 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Note the basophilic neoplastic cells arranged in sheets with palisading around vascular spaces. Nuclei are generally irregularly oval to round with a scant amount of basophilic cytoplasm; mitotic figures are numerous (white arrows). H&E

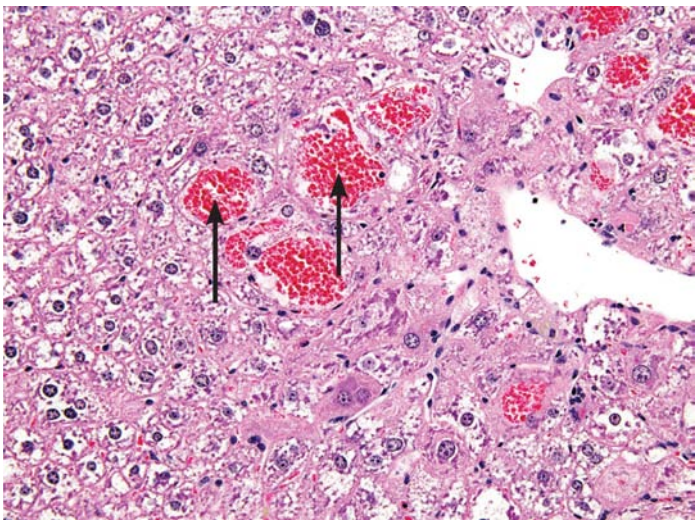


PLATE 14
Erythrophagocytosis in the liver of a male B6C3F1/N mouse administered 200 mg/kg *Ginkgo biloba* extract by gavage for 2 years. The erythrophagocytosis is characterized by enlarged hepatocytes in which the cytoplasm is filled with red blood cells (arrows). H&E

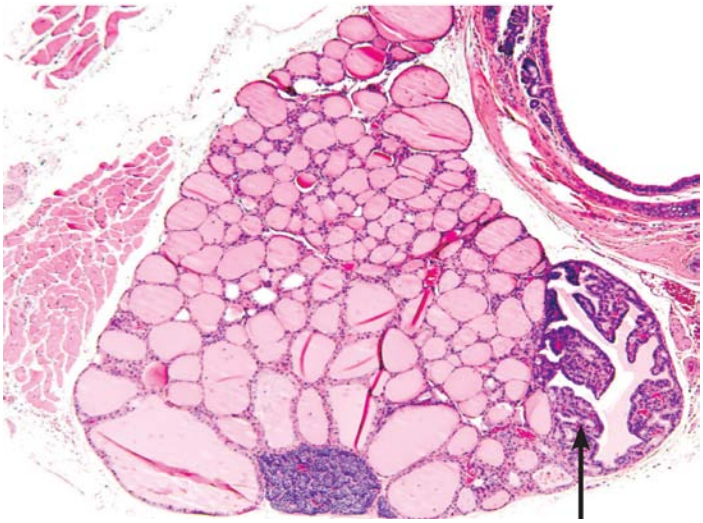


PLATE 15
Follicular cell adenoma in the thyroid gland of a male B6C3F1/N mouse administered 2,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Note the well circumscribed nonencapsulated mass formed by the proliferation of follicular cells within the thyroid gland. The neoplastic cells form papillary projections with complex branching protruding into lumens (arrow). H&E

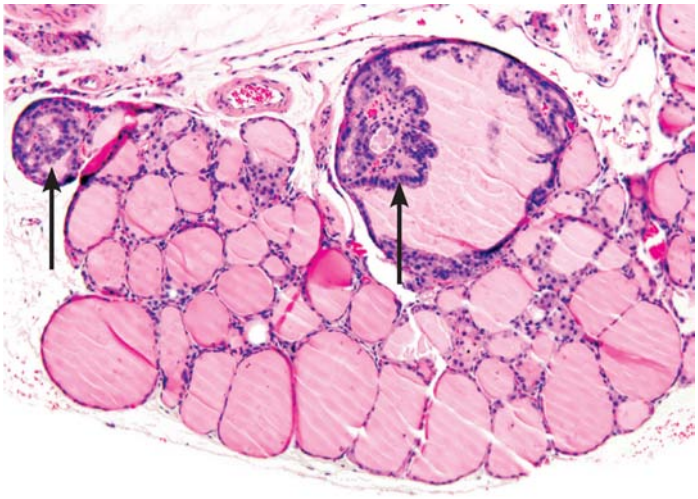


PLATE 16
Follicle hyperplasia in the thyroid gland of a male B6C3F1/N mouse administered 2,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. The hyperplastic nodules (arrows) are characterized by follicles lined by a crowded cuboidal to low columnar single cell layer epithelium. The right nodule is enlarged due to increased colloid. H&E

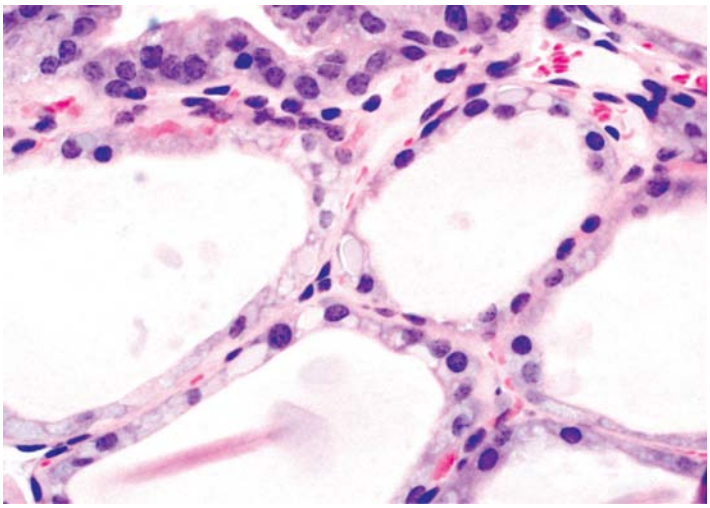


PLATE 17
Follicular cell hypertrophy in the thyroid gland of a male B6C3F1/N mouse administered 2,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. The follicles are lined by tall cuboidal epithelial cells, which are one to two times larger than normal follicle epithelial cells. These cells often contain yellow to pink globules in the cytoplasm or fine vacuoles. H&E

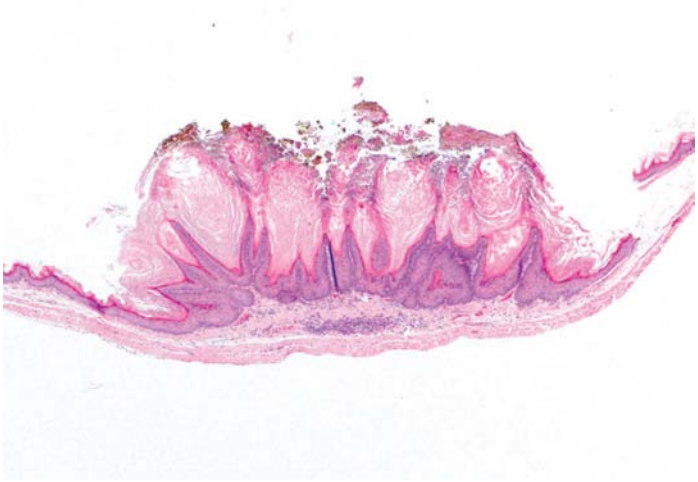


PLATE 18
Epithelium hyperplasia in the forestomach of a female B6C3F1/N mouse administered 2,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Note the focal presence of finger-like projections, each with its own lamina propria, oriented perpendicularly to the basal lamina. H&E

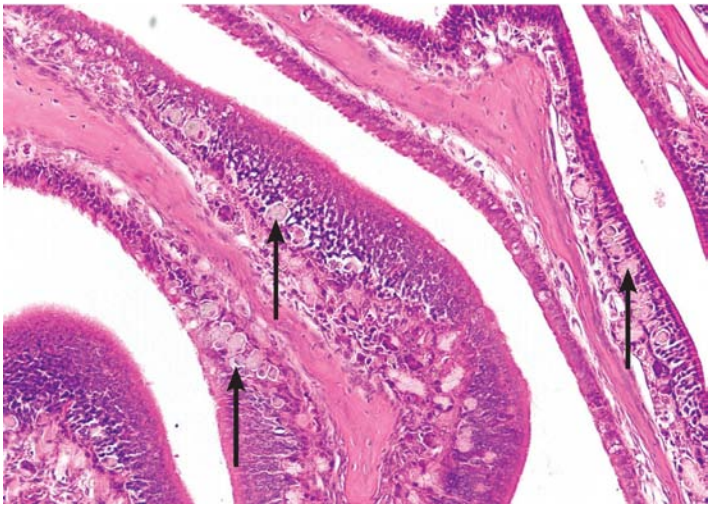


PLATE 19
Pigmentation in the olfactory epithelium of the nose (Level III) in a male B6C3F1/N mouse administered 2,000 mg/kg *Ginkgo biloba* extract by gavage for 2 years. Note the presence of macrophages with an abundance of golden brown granular cytoplasm usually located immediately above the basal cell layer and below the nuclear layer of the sustentacular cells and olfactory neurons (arrows). H&E

DISCUSSION AND CONCLUSIONS

Ginkgo biloba extract is a popular herbal supplement used to improve brain function. As with many natural products, there is significant variability in the contents of *Ginkgo biloba* extract available in the marketplace (Kressmann *et al.*, 2002; Agnolet *et al*., 2010; Gawron-Gzella *et al*., 2010; Chandra *et al*., 2011). In a 2002 study analyzing *Ginkgo biloba* extract constituents from products available in the United States, Kressmann *et al*. (2002) found a range of concentrations for flavonol glycosides (24% to 36%), terpene lactones (4% to 11%), and ginkgolic acids (less than 500 ppm to 90,000 ppm). The *Ginkgo biloba* extract used in the present studies contained 31.2% flavonol glycosides, 15.4% terpene lactones (6.94% bilobalide, 3.74% ginkgolide A, 1.62% ginkgolide B, 3.06% ginkgolide C), and 10.45 ppm ginkgolic acid. These values reflect concentrations measured in commercially available products in the United States and have a similar ratio of active ingredients to the standardized *Ginkgo biloba* extract (EGb 761®).

In male and female rats and mice, the liver, thyroid gland, and nose were the major targets of *Ginkgo biloba* extract toxicity. Many of the toxic and carcinogenic effects associated with *Ginkgo biloba* extract administration are characteristic of lesions related to hepatic enzyme induction. In general, the carcinogenic activity of *Ginkgo biloba* extract was more pronounced in mice than rats.

A variety of liver lesions occurred in rats and mice following *Ginkgo biloba* extract administration. At 3 months, *Ginkgo biloba* extract caused increased liver weight, centrilobular hepatocytic hypertrophy, minimal focal necrosis (male mice) or fatty change of hepatocytes (male rats). These findings are consistent with changes associated with hepatic enzyme induction (Maronpot *et al*., 2010). Allen *et al.* (2004) retrospectively analyzed the relative predictive value of these 3-month parameters on 2-year liver tumor outcomes and concluded that hepatocellular hypertrophy and increased liver weight accompanied by degenerative changes (i.e. hepatocyte necrosis, vacuolization and/or fatty change) were significantly predictive of hepatocellular neoplasm development in the mouse or rat.

Liver carcinogenicity was evident following 2 years of *Ginkgo biloba* extract administration in male and female mice, and to a lesser extent in male rats. In male and female mice, the incidences of hepatocellular carcinoma and hepatoblastoma increased with dose, and in female mice, incidences of hepatocellular adenoma also increased with dose. Additionally, the incidences of multiple hepatocellular carcinomas and hepatoblastomas in male and female mice and of multiple adenomas in female mice increased in a dose-dependent manner. These findings were considered clear evidence for carcinogenic activity of *Ginkgo biloba* extract in male and female mice.

Hepatoblastomas are relatively rare tumors in mice, although they can occur spontaneously or be induced by various chemicals (Turusov *et al*., 2002). The incidence rate of hepatoblastoma in mice administered *Ginkgo biloba* extract is among the highest generated from chemical exposures in NTP studies to date (Turusov *et al.*, 2002). There is evidence that the development of hepatoblastomas in a mouse model (D2B6F1) is mediated by promoting agents, rather than initiating agents (Diwan *et al*., 1995). Furthermore, the prototypical promoting agent phenobarbital, as well as other enzyme inducing compounds, oxazepam and primidone, elicit significant increases in hepatoblastomas in mice (Bucher *et al*., 1994; Diwan *et al*., 1995; NTP, 2000).

In male rats, the incidences of hepatocellular adenoma in the 100 and 300 mg/kg groups were above the historical control range for corn oil gavage studies. It was concluded that the hepatocellular adenomas in male rats may have been related to *Ginkgo biloba* extract administration.

Many nonneoplastic effects were observed in the livers of rats and mice administered *Ginkgo biloba* extract for 2 years. In rats, 2-year *Ginkgo biloba* extract administration was associated with increased incidences of centrilobular hepatocytic hypertrophy and bile duct hyperplasia in males and females; cystic degeneration, oval cell hyperplasia, and hepatocellular necrosis in males; and focal fatty change in females. In mice, *Ginkgo biloba* extract administration was associated with hepatocytic hypertrophy and hepatocytic erythrophagocytosis in males and females; hematopoietic cell proliferation, inflammation, and necrosis in males; and increased eosinophilic and mixed cell foci of altered hepatocytes in females.

The thyroid gland was another important target for *Ginkgo biloba* extract toxicity and the pathology at this site is consistent with a hepatic microsomal enzyme induction mechanism of toxicity. Increased thyroid stimulating hormone (TSH) levels were observed in all male dosed groups and the high dose female special study rats following 14 weeks of *Ginkgo biloba* extract administration. In the 3-month rat study, follicular cell hypertrophy was observed in dosed male and female rats.

In the 2-year studies, neoplastic findings included incidences of thyroid gland follicular cell adenoma in male and female rats and male mice above the historical control incidences for corn oil gavage studies. Additionally, in the 300 and 1,000 mg/kg groups of female rats there were single occurrences of follicular cell carcinoma, which is a rare neoplasm observed in only 5 of 1,186 (0.4%) historical control female rats from studies representing all routes of administration. The occurrence of these neoplasms, as well as the support from nonneoplastic lesions (follicular cell hypertrophy in male and female rats and mice and follicular cell hyperplasia in male rats and mice) and elevated TSH levels in treated male and female rats indicated that follicular cell neoplasms in male and female rats and male mice were related to *Ginkgo biloba* administration.

The third target of *Ginkgo biloba* extract toxicity was the nose. In the 3-month studies, atrophy of the olfactory epithelium was observed in male and female mice. Hyaline droplet accumulation of the respiratory epithelium and olfactory epithelium were noted in mice. Additionally, there were dose-related increases in pigmentation of the macrophages in the olfactory epithelium in male and female rats and mice. In the 2-year study, neoplasms were confined to two respiratory epithelial adenomas at the middle dose (300 mg/kg) of *Ginkgo biloba* extract in female rats. This is a rare neoplasm in rats, occurring in only 1 of 1,196 (0.1%) female rats in the historical controls for all routes of administration. A conclusion of equivocal evidence of carcinogenic activity was based on the rarity of this tumor and the supporting nonneoplastic lesions discussed below.

Nonneoplastic lesions observed in the nose of male and female rats in the 2-year study included transitional epithelium hyperplasia, respiratory epithelium hyperplasia, olfactory epithelium atrophy, respiratory metaplasia, nerve atrophy, and pigmentation. In mice in the 2-year study, there were increased incidences of hyaline droplet accumulation and pigmentation in the olfactory epithelium.

The pathogenesis of nasal lesions associated with *Ginkgo biloba* extract administration could be related to systemic exposure to *Ginkgo biloba* extract or its metabolites (Sells *et al*., 2007). It is of note that the CYP450 concentration in the rat olfactory epithelium is second only to the liver (Reed, 1993). Furthermore, in both studies, the olfactory epithelium was more severely affected compared to the respiratory epithelium, which corresponds to its higher levels of enzymatic activity (Walsh and Courtney, 1998).

In addition to the major targets of *Ginkgo biloba* extract toxicity (liver, thyroid gland, and nose) that were consistent across species and sex, male rats had dose-related increased incidences of mononuclear cell leukemia, one of the most commonly reported lesions in aging Fisher rats (Stromberg and Vogstberger, 1983). The 18% rate observed in vehicle control male rats in the current study is consistent with the historical control rate for corn oil gavage studies (range of 8% to 28% for combined lymphocytic, monocytic, mononuclear, or undifferentiated leukemias in F344/N male rats). The significant dose-dependent increases reached 44% and 42% in the 300 and 1,000 mg/kg groups, respectively. Therefore, the increase in mononuclear cell leukemia in male rats may have been related to *Ginkgo biloba* extract administration. However, due to the variability in the occurrence of this lesion in historical control animals across all routes (36% ± 14%; range of 8% to 58%), the increased incidence in this study could not be definitively attributed to *Ginkgo biloba* extract administration.

In the present studies, there was a dose-related increase in the severity of nephropathy in male rats. However, significant increases in incidences of renal neoplasms were not noted in treated male rats. This contrasts with findings from a previous 2-year study with quercetin, in which *some evidence* of carcinogenic activity in male rats was based on an increased incidence of renal tubule cell adenoma (NTP, 1992). Although *Ginkgo biloba* extract contains quercetin, the levels of quercetin in the current study were well below the high dose of quercetin (40,000 ppm) that induced renal neoplasms in male rats (NTP, 1992).

Ginkgo biloba extract was shown to be clearly genotoxic in bacterial mutagenicity assays conducted by the NTP (Table E1). Positive responses in such assays are considered to be strong alerts to carcinogenic activity in rodents, and indeed, tumor induction was observed in the studies reported here. The flavonol glycosides, quercetin and

kaempferol, present in *Ginkgo biloba* extract, are demonstrated mutagens in a variety of *in vitro* test systems and could contribute to the genotoxicity observed with the extract.

A companion gene expression study was conducted to aid in identifying a mechanism of tumorigenicity for *Ginkgo biloba* extract. Microarray and supporting polymerase chain reaction assessments were performed on RNA samples from liver tissue of male mice in the 2-year study (Guo *et al*., 2010). Male mice exposed to 2,000 mg/kg *Ginkgo biloba* extract for 2 years displayed differential expression of 68 out of a possible 313 drug metabolizing enzymes present on the array (i.e., 22%). Pathway analysis revealed the top canonical pathway to be "metabolism of xenobiotics by P450." Additionally, the Myc-centered network was identified as an important target of *Ginkgo biloba* extract. Myc is a proto-oncogene involved in cell growth, proliferation, differentiation, and apoptosis that has been found to cooperate with non-genotoxic hepatotoxicants to accelerate the development of liver tumors in mice (Beer *et al*., 2008).

There is concern that the modulation of drug metabolizing enzymes by herbal products could lead to significant drug-herb interactions (Sparreboom *et al*., 2004). *In vitro* studies conducted in support of the NTP assays examined the effects of *Ginkgo biloba* extract (and its hydrolyzed derivative) on the activity of xenobiotic metabolizing enzymes in human hepatocytes (Etheridge *et al*., 2007). Several cytochrome P450s (1A2, 2C8, 2C9, and 3A4) exhibited decreased activity. Lending further support to potential drug-herb interactions of *Ginkgo biloba* extract, Robertson *et al*. (2008) found that *Ginkgo biloba* extract treatment significantly reduced the area under the curve and C_{max} of midazolam, and Shinozuka *et al*. (2002) found decreased efficacy of the hypotensive drug nicardipine following 4 weeks of *Ginkgo biloba* extract treatment in rats.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity** of *Ginkgo biloba* extract in male F344/N rats based on increased incidences of thyroid gland follicular cell adenoma. The increased incidences of mononuclear cell leukemia and hepatocellular adenoma may have been related to *Ginkgo biloba* extract administration. There was *some evidence of carcinogenic activity* of *Ginkgo biloba* extract in female F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms. Increased occurrence of

respiratory epithelium adenomas in the nose may have been related to *Ginkgo biloba* extract administration. There was *clear evidence of carcinogenic activity* of *Ginkgo biloba* extract in male B6C3F1/N mice based on increased incidences of hepatocellular carcinoma and hepatoblastoma. The increased incidences of thyroid gland follicular cell adenoma were also related to *Ginkgo biloba* extract administration. There was *clear evidence of carcinogenic activity* of *Ginkgo biloba* extract in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma.

Administration of *Ginkgo biloba* extract resulted in increased incidences of nonneoplastic lesions in the liver, thyroid gland, and nose of male and female rats and mice and the forestomach of male and female mice. Increased severity of nephropathy in male rats was also due to administration of *Ginkgo biloba* extract.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 15.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR GAVAGE STUDY OF *GINKGO BILOBA* EXTRACT

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	7	8	10	14
Natural deaths	5	5	9	20
Survivors				
Terminal kill	38	37	31	16
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(49)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(49)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Leiomyoma	1 (2%)			
Schwannoma malignant		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma		2 (4%)	3 (6%)	
Hepatocellular adenoma, multiple		1 (2%)		
Ito cell tumor benign		1 (2%)		
Osteosarcoma, metastatic, bone			1 (2%)	
Mesentery	(4)	(4)	(8)	(5)
Hemangioma		1 (25%)		
Hemangiosarcoma			1 (13%)	
Pancreas	(50)	(50)	(50)	(50)
Acinus, adenoma				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	11 (22%)	4 (8%)	4 (8%)	6 (12%)
Pheochromocytoma benign, multiple				1 (2%)
Pheochromocytoma complex				1 (2%)
Pheochromocytoma malignant	1 (2%)	1 (2%)		1 (2%)
Bilateral, pheochromocytoma benign		2 (4%)	1 (2%)	
Bilateral, pheochromocytoma malignant	1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	2 (4%)	1 (2%)	1 (2%)
Carcinoma			1 (2%)	1 (2%)
Parathyroid gland	(47)	(48)	(48)	(47)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	38 (76%)	28 (56%)	25 (50%)	18 (36%)
Pars distalis, adenoma, multiple		2 (4%)		1 (2%)
Pars distalis, carcinoma				1 (2%)
Pars intermedia, adenoma	1 (2%)	1 (2%)		1 (2%)
Thyroid gland	(50)	(50)	(49)	(45)
C-cell, adenoma	10 (20%)	9 (18%)	10 (20%)	1 (2%)
C-cell, carcinoma	1 (2%)	2 (4%)	2 (4%)	
Follicle, adenoma	2 (4%)	1 (2%)	3 (6%)	5 (11%)
General Body System				
None				
Genital System				
Coagulating gland	(0)	(0)	(1)	(0)
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	3 (6%)		3 (6%)
Carcinoma			1 (2%)	2 (4%)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	26 (52%)	29 (58%)	26 (52%)	27 (54%)
Interstitial cell, adenoma	11 (22%)	8 (16%)	13 (26%)	12 (24%)
Hematopoietic System				
Bone marrow	(50)	(50)	(48)	(50)
Lymph node	(2)	(2)	(9)	(10)
Mediastinal, carcinoma, metastatic, thyroid gland			1 (11%)	
Lymph node, mandibular	(0)	(0)	(1)	(0)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma			2 (4%)	
Osteosarcoma, metastatic, bone			1 (2%)	
Thymus	(47)	(50)	(47)	(47)
Thymoma benign	1 (2%)			1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibroadenoma	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	1 (2%)	1 (2%)	
Keratoacanthoma	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Keratoacanthoma, multiple	1 (2%)			1 (2%)
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Subcutaneous tissue, fibrosarcoma				3 (6%)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)			
Subcutaneous tissue, schwannoma malignant	1 (2%)		2 (4%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma			2 (4%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(0)	(0)	(0)	(1)
Spinal cord	(0)	(0)	(0)	(1)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Alveolar/bronchiolar carcinoma			1 (2%)	1 (2%)
Carcinoma, metastatic, thyroid gland	1 (2%)	1 (2%)	1 (2%)	
Cystic keratinizing epithelioma			1 (2%)	
Osteosarcoma, metastatic, bone			1 (2%)	
Pheochromocytoma malignant, metastatic, adrenal medulla	1 (2%)	1 (2%)		
Nose	(50)	(49)	(49)	(50)
Pleura	(0)	(0)	(1)	(0)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(1)	(0)	(0)	(1)
Carcinoma	1 (100%)			1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Lipoma	1 (2%)			
Renal tubule, adenoma			1 (2%)	1 (2%)
Transitional epithelium, carcinoma		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	9 (18%)	12 (24%)	22 (44%)	21 (42%)
Mesothelioma malignant	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	48	50	49
Total primary neoplasms	135	120	131	121
Total animals with benign neoplasms	50	48	49	48
Total benign neoplasms	117	101	96	88
Total animals with malignant neoplasms	13	17	30	29
Total malignant neoplasms	18	19	35	33
Total animals with metastatic neoplasms	3	3	2	
Total metastatic neoplasms	3	3	5	

[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
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	11/50 (22%)	6/50 (12%)	5/50 (10%)	7/50 (14%)
Adjusted rate[b]	23.6%	13.0%	11.1%	16.4%
Terminal rate[c]	9/38 (24%)	4/37 (11%)	3/31 (10%)	2/16 (13%)
First incidence (days)	699	662	662	668
Poly-3 test[d]	P=0.423N	P=0.146N	P=0.095N	P=0.283N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	12/50 (24%)	7/50 (14%)	5/50 (10%)	9/50 (18%)
Adjusted rate	25.7%	15.2%	11.1%	21.1%
Terminal rate	10/38 (26%)	5/37 (14%)	3/31 (10%)	3/16 (19%)
First incidence (days)	699	662	662	668
Poly-3 test	P=0.564	P=0.157N	P=0.060N	P=0.396N
Liver: Hepatocellular Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	6.5%	6.7%	0.0%
Terminal rate	0/38 (0%)	2/37 (5%)	2/31 (7%)	0/16 (0%)
First incidence (days)	—[e]	714	692	—
Poly-3 test	P=0.325N	P=0.117	P=0.112	—[f]
Mammary Gland: Fibroadenoma				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.3%	2.2%	6.7%	2.4%
Terminal rate	2/38 (5%)	0/37 (0%)	1/31 (3%)	0/16 (0%)
First incidence (days)	727 (T)	706	626	665
Poly-3 test	P=0.504N	P=0.504N	P=0.486	P=0.534N
Pancreatic Islets: Adenoma				
Overall rate	4/50 (8%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	8.6%	4.4%	2.2%	2.4%
Terminal rate	3/38 (8%)	2/37 (5%)	1/31 (3%)	0/16 (0%)
First incidence (days)	626	727 (T)	727 (T)	689
Poly-3 test	P=0.211N	P=0.346N	P=0.194N	P=0.213N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	2/50 (4%)	2/50 (4%)	2/50 (4%)
Adjusted rate	8.6%	4.4%	4.5%	4.7%
Terminal rate	3/38 (8%)	2/37 (5%)	1/31 (5%)	0/16 (0%)
First incidence (days)	626	727 (T)	722	591
Poly-3 test	P=0.421N	P=0.346N	P=0.360N	P=0.383N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	38/50 (76%)	30/50 (60%)	25/50 (50%)	19/50 (38%)
Adjusted rate	76.4%	61.8%	53.5%	41.9%
Terminal rate	28/38 (74%)	21/37 (57%)	18/31 (58%)	3/16 (19%)
First incidence (days)	505	449	534	568
Poly-3 test	P<0.001N	P=0.087N	P=0.013N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	38/50 (76%)	30/50 (60%)	25/50 (50%)	20/50 (40%)
Adjusted rate	76.4%	61.8%	53.5%	43.5%
Terminal rate	28/38 (74%)	21/37 (57%)	18/31 (58%)	3/16 (19%)
First incidence (days)	505	449	534	510
Poly-3 test	P<0.001N	P=0.087N	P=0.013N	P<0.001N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Preputial Gland: Adenoma				
Overall rate	4/50 (8%)	3/50 (6%)	0/49 (0%)	3/50 (6%)
Adjusted rate	8.6%	6.5%	0.0%	7.0%
Terminal rate	4/38 (11%)	2/37 (5%)	0/30 (0%)	0/16 (0%)
First incidence (days)	727 (T)	662	—	600
Poly-3 test	P=0.573N	P=0.504N	P=0.069N	P=0.545N
Preputial Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	1/49 (2%)	5/50 (10%)
Adjusted rate	8.6%	6.5%	2.3%	11.6%
Terminal rate	4/38 (11%)	2/37 (5%)	0/30 (0%)	1/16 (6%)
First incidence (days)	727 (T)	662	722	600
Poly-3 test	P=0.274	P=0.504N	P=0.198N	P=0.452
Skin: Keratoacanthoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	4/50 (8%)
Adjusted rate	4.3%	6.5%	2.2%	9.4%
Terminal rate	2/38 (5%)	3/37 (8%)	1/31 (3%)	2/16 (13%)
First incidence (days)	727 (T)	727 (T)	727 (T)	602
Poly-3 test	P=0.234	P=0.494	P=0.514N	P=0.296
Skin: Keratoacanthoma or Basal Cell Adenoma				
Overall rate	2/50 (4%)	4/50 (8%)	2/50 (4%)	4/50 (8%)
Adjusted rate	4.3%	8.7%	4.5%	9.4%
Terminal rate	2/38 (5%)	4/37 (11%)	2/31 (7%)	2/16 (13%)
First incidence (days)	727 (T)	727 (T)	727 (T)	602
Poly-3 test	P=0.320	P=0.332	P=0.679	P=0.296
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	7.0%
Terminal rate	0/38 (0%)	0/37 (0%)	0/31 (0%)	1/16 (6%)
First incidence (days)	—	—	—	505
Poly-3 test	P=0.007	—	—	P=0.106
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Fibrosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.2%	0.0%	0.0%	7.0%
Terminal rate	0/38 (0%)	0/37 (0%)	0/31 (0%)	1/16 (6%)
First incidence (days)	684	—	—	505
Poly-3 test	P=0.047	P=0.503N	P=0.509N	P=0.278
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, or Fibrosarcoma				
Overall rate	2/50 (4%)	1/50 (2%)	1/50 (2%)	4/50 (8%)
Adjusted rate	4.3%	2.2%	2.2%	9.2%
Terminal rate	1/38 (3%)	1/37 (3%)	0/31 (0%)	1/16 (6%)
First incidence (days)	684	727 (T)	588	505
Poly-3 test	P=0.111	P=0.505N	P=0.512N	P=0.305
Testes: Adenoma				
Overall rate	37/50 (74%)	37/50 (74%)	39/50 (78%)	39/50 (78%)
Adjusted rate	79.1%	79.1%	81.6%	81.3%
Terminal rate	34/38 (90%)	31/37 (84%)	25/31 (81%)	15/16 (94%)
First incidence (days)	692	657	547	505
Poly-3 test	P=0.450	P=0.602N	P=0.482	P=0.498

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Thyroid Gland (C-Cell): Adenoma				
Overall rate	10/50 (20%)	9/50 (18%)	10/49 (20%)	1/45 (2%)
Adjusted rate	21.3%	19.6%	22.8%	2.7%
Terminal rate	9/38 (24%)	9/37 (24%)	9/31 (29%)	0/16 (0%)
First incidence (days)	526	727 (T)	662	665
Poly-3 test	P=0.012N	P=0.525N	P=0.532	P=0.012N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	11/50 (22%)	11/50 (22%)	12/49 (24%)	1/45 (2%)
Adjusted rate	23.4%	24.0%	27.3%	2.7%
Terminal rate	10/38 (26%)	11/37 (30%)	10/31 (32%)	0/16 (0%)
First incidence (days)	526	727 (T)	662	665
Poly-3 test	P=0.006N	P=0.569	P=0.426	P=0.007N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	2/50 (4%)	1/50 (2%)	3/49 (6%)	5/45 (11%)
Adjusted rate	4.3%	2.2%	6.7%	13.2%
Terminal rate	2/38 (5%)	0/37 (0%)	1/31 (3%)	2/16 (13%)
First incidence (days)	727 (T)	685	485	675
Poly-3 test	P=0.040	P=0.503N	P=0.482	P=0.142
All Organs: Hemangiosarcoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	6.7%	0.0%
Terminal rate	0/38 (0%)	0/37 (0%)	2/31 (7%)	0/16 (0%)
First incidence (days)	—	—	664	—
Poly-3 test	P=0.663N	—	P=0.112	—
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	2.2%	6.7%	0.0%
Terminal rate	0/38 (0%)	0/37 (0%)	2/31 (7%)	0/16 (0%)
First incidence (days)	—	657	664	—
Poly-3 test	P=0.524N	P=0.499	P=0.112	—
All Organs: Mononuclear Cell Leukemia				
Overall rate	9/50 (18%)	12/50 (24%)	22/50 (44%)	21/50 (42%)
Adjusted rate	19.2%	25.7%	45.7%	46.8%
Terminal rate	6/38 (16%)	7/37 (19%)	9/31 (29%)	7/16 (44%)
First incidence (days)	626	643	485	568
Poly-3 test	P=0.004	P=0.308	P=0.004	P=0.003
All Organs: Malignant Mesothelioma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.4%	4.4%	2.2%	2.4%
Terminal rate	2/38 (5%)	2/37 (5%)	1/31 (3%)	0/16 (0%)
First incidence (days)	684	727 (T)	727 (T)	675
Poly-3 test	P=0.303N	P=0.507N	P=0.321N	P=0.343N
All Organs: Benign Neoplasms				
Overall rate	50/50 (100%)	48/50 (96%)	49/50 (98%)	48/50 (96%)
Adjusted rate	100.0%	98.0%	98.3%	96.2%
Terminal rate	38/38 (100%)	36/37 (100%)	31/31 (100%)	15/16 (94%)
First incidence (days)	505	449	485	505
Poly-3 test	P=0.209N	P=0.496N	P=0.569N	P=0.249N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
All Organs: Malignant Neoplasms				
Overall rate	13/50 (26%)	17/50 (34%)	30/50 (60%)	29/50 (58%)
Adjusted rate	27.5%	36.2%	61.1%	61.5%
Terminal rate	8/38 (21%)	11/37 (30%)	14/31 (45%)	10/16 (63%)
First incidence (days)	626	643	485	505
Poly-3 test	P<0.001	P=0.248	P<0.001	P<0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	48/50 (96%)	50/50 (100%)	49/50 (98%)
Adjusted rate	100.0%	98.0%	100.0%	98.2%
Terminal rate	38/38 (100%)	36/37 (100%)	31/31 (100%)	16/16 (100%)
First incidence (days)	505	449	485	505
Poly-3 test	P=0.509N	P=0.496N	—	P=0.542N

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value 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 Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE A3a
Historical Incidence of Thyroid Gland Follicular Cell Adenoma in Control Male F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
Ginkgo biloba extract (March 2005)	2/50
Isoeugenol (April 2002)	1/50
Kava kava extract (August 2004)	1/49
β-Myrcene (March 2002)	1/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	1/50
Pulegone (April 2003)	0/50
Total (%)	6/299 (2.0%)
Mean ± standard deviation	2.0% ± 1.3%
Range	0%-4%
Overall Historical Incidence: All Routes	
Total (%)	13/1,239 (1.1%)
Mean ± standard deviation	1.0% ± 1.7%
Range	0%-6%

[a] Data as of May 2011

TABLE A3b
Historical Incidence of Mononuclear Cell Leukemia in Control Male F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
Ginkgo biloba extract (March 2005)	9/50
Isoeugenol (April 2002)	10/50
Kava kava extract (August 2004)	7/49
β-Myrcene (March 2002)	9/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	14/50
Pulegone (April 2003)	4/50
Total (%)	53/299 (17.7%)
Mean ± standard deviation	17.7% ± 6.6%
Range	8%-28%
Overall Historical Incidence: All Routes	
Total (%)	450/1,249 (36.0%)
Mean ± standard deviation	36.0% ± 14.4%
Range	8%-58%

[a] Data as of May 2011; may include lymphocytic, monocytic, mononuclear, or undifferentiated leukemia.

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	7	8	10	14
Natural deaths	5	5	9	20
Survivors				
Terminal kill	38	37	31	16
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(49)	(50)	(50)
Inflammation, chronic				1 (2%)
Thrombosis	1 (2%)			
Ulcer	1 (2%)			
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan			2 (4%)	1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Inflammation, acute				1 (2%)
Parasite metazoan	3 (6%)	8 (16%)	5 (10%)	5 (10%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(49)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)	5 (10%)	2 (4%)	3 (6%)
Basophilic focus	30 (60%)	37 (74%)	23 (46%)	22 (44%)
Clear cell focus	31 (62%)	30 (60%)	18 (36%)	11 (22%)
Congestion	1 (2%)			
Degeneration, cystic	4 (8%)	14 (28%)	10 (20%)	14 (28%)
Eosinophilic focus	14 (28%)	21 (42%)	19 (38%)	21 (42%)
Fatty change, focal	3 (6%)			
Fibrosis		1 (2%)		
Hepatodiaphragmatic nodule	2 (4%)	3 (6%)	3 (6%)	
Inflammation, chronic	44 (88%)	41 (82%)	30 (60%)	32 (64%)
Mixed cell focus	19 (38%)	32 (64%)	24 (48%)	17 (34%)
Necrosis	1 (2%)	4 (8%)	6 (12%)	7 (14%)
Thrombosis				1 (2%)
Bile duct, hyperplasia	32 (64%)	43 (86%)	46 (92%)	46 (92%)
Hepatocyte, fatty change	27 (54%)	18 (36%)	23 (46%)	31 (62%)
Hepatocyte, hypertrophy	1 (2%)	17 (34%)	26 (52%)	27 (54%)
Oval cell, hyperplasia		1 (2%)	1 (2%)	10 (20%)
Mesentery	(4)	(4)	(8)	(5)
Thrombosis				1 (20%)
Fat, necrosis	2 (50%)	2 (50%)	5 (63%)	3 (60%)
Pancreas	(50)	(50)	(50)	(50)
Inflammation, chronic		1 (2%)	1 (2%)	
Mineralization	1 (2%)			
Necrosis				1 (2%)
Acinus, atrophy	22 (44%)	23 (46%)	14 (28%)	20 (40%)
Acinus, hyperplasia	11 (22%)	7 (14%)	5 (10%)	12 (24%)
Duct, cyst	3 (6%)	5 (10%)	10 (20%)	3 (6%)
Salivary glands	(50)	(50)	(50)	(50)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic	4 (8%)	1 (2%)	6 (12%)	6 (12%)
Mineralization			1 (2%)	
Ulcer	2 (4%)		4 (8%)	4 (8%)
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)		
Inflammation, chronic		1 (2%)	1 (2%)	
Mineralization	1 (2%)	1 (2%)	1 (2%)	6 (12%)
Ulcer	2 (4%)			2 (4%)
Epithelium, hyperplasia	1 (2%)			
Glands, ectasia	17 (34%)	30 (60%)	19 (38%)	12 (24%)
Glands, hyperplasia		2 (4%)		1 (2%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Thrombosis				1 (2%)
Aorta, mineralization	1 (2%)			2 (4%)
Pulmonary vein, mineralization	1 (2%)			
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	50 (100%)	48 (96%)	49 (98%)	50 (100%)
Atrium, thrombosis	3 (6%)		1 (2%)	3 (6%)
Myocardium, mineralization	1 (2%)			2 (4%)
Valve, inflammation, chronic			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Cytoplasmic alteration		1 (2%)		
Degeneration, cystic	2 (4%)	4 (8%)		
Hyperplasia, focal	12 (24%)	9 (18%)	9 (18%)	8 (16%)
Hypertrophy, focal	7 (14%)	10 (20%)	9 (18%)	3 (6%)
Hypertrophy, diffuse	3 (6%)	3 (6%)	5 (10%)	11 (22%)
Infiltration cellular, mononuclear cell		1 (2%)		
Inflammation, chronic				1 (2%)
Necrosis		1 (2%)	2 (4%)	1 (2%)
Vacuolization cytoplasmic, focal	17 (34%)	17 (34%)	16 (32%)	13 (26%)
Vacuolization cytoplasmic, diffuse	18 (36%)	25 (50%)	33 (66%)	34 (68%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	15 (30%)	16 (32%)	8 (16%)	10 (20%)
Necrosis			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	2 (4%)	5 (10%)	2 (4%)
Parathyroid gland	(47)	(48)	(48)	(47)
Hyperplasia	1 (2%)	3 (6%)	2 (4%)	7 (15%)
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	1 (2%)
Cyst	5 (10%)	4 (8%)	7 (14%)	8 (16%)
Pars distalis, hyperplasia	19 (38%)	18 (36%)	18 (36%)	16 (32%)
Pars intermedia, hyperplasia	1 (2%)			1 (2%)
Thyroid gland	(50)	(50)	(49)	(45)
C-cell, hyperplasia	35 (70%)	29 (58%)	27 (55%)	24 (53%)
Follicle, hyperplasia		7 (14%)	9 (18%)	5 (11%)
Follicular cell, hypertrophy	13 (26%)	37 (74%)	41 (84%)	41 (91%)
General Body System				
None				

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Genital System				
Coagulating gland	(0)	(0)	(1)	(0)
Inflammation, chronic			1 (100%)	
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm		1 (2%)	3 (6%)	
Inflammation, chronic				1 (2%)
Mineralization		1 (2%)		
Preputial gland	(50)	(50)	(50)	(50)
Cyst	2 (4%)			
Hyperplasia	2 (4%)	1 (2%)		
Inflammation, chronic	49 (98%)	48 (96%)	47 (94%)	49 (98%)
Metaplasia, cartilagenous			1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Hyperplasia	6 (12%)	3 (6%)	3 (6%)	4 (8%)
Inflammation	36 (72%)	27 (54%)	25 (50%)	32 (64%)
Necrosis	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	3 (6%)	2 (4%)	4 (8%)
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, atrophy	1 (2%)		1 (2%)	1 (2%)
Germinal epithelium, necrosis			1 (2%)	
Interstitial cell, hyperplasia				1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(48)	(50)
Hyperplasia	14 (28%)	15 (30%)	21 (44%)	26 (52%)
Necrosis			1 (2%)	
Lymph node	(2)	(2)	(9)	(10)
Deep cervical, hyperplasia, plasma cell				1 (10%)
Mediastinal, ectasia			1 (11%)	1 (10%)
Mediastinal, hyperplasia, lymphoid		1 (50%)	1 (11%)	3 (30%)
Mediastinal, hyperplasia, plasma cell				1 (10%)
Mediastinal, infiltration cellular, histiocyte		1 (50%)		
Mediastinal, inflammation, suppurative	1 (50%)			
Pancreatic, necrosis				1 (10%)
Renal, hyperplasia, lymphoid				1 (10%)
Lymph node, mandibular	(0)	(0)	(1)	(0)
Ectasia			1 (100%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia			1 (2%)	
Hyperplasia, lymphoid		1 (2%)		
Infiltration cellular, histiocyte	1 (2%)	1 (2%)		
Necrosis		1 (2%)	1 (2%)	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)	1 (2%)		
Hematopoietic cell proliferation	3 (6%)	8 (16%)	4 (8%)	6 (12%)
Hemorrhage		1 (2%)		
Necrosis		1 (2%)	1 (2%)	1 (2%)
Lymphoid follicle, atrophy	2 (4%)	1 (2%)	4 (8%)	14 (28%)
Thymus	(47)	(50)	(47)	(47)
Atrophy	47 (100%)	48 (96%)	46 (98%)	48 (98%)
Epithelial cell, hyperplasia	1 (2%)	1 (2%)		1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Inflammation, chronic		1 (2%)		
Duct, cyst	12 (24%)	19 (38%)	13 (26%)	12 (24%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Lymphatic, subcutaneous tissue, cyst	1 (2%)	1 (2%)		
Sebaceous gland, cyst	1 (2%)			
Subcutaneous tissue, hemorrhage				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis		1 (2%)	1 (2%)	1 (2%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Necrosis		1 (2%)		
Hippocampus, necrosis				1 (2%)
Peripheral nerve	(0)	(0)	(0)	(1)
Axon, degeneration				1 (100%)
Spinal cord	(0)	(0)	(0)	(1)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Inflammation, acute				1 (2%)
Inflammation, chronic	19 (38%)	14 (28%)	12 (24%)	8 (16%)
Metaplasia, osseous	1 (2%)	1 (2%)	2 (4%)	
Thrombosis			1 (2%)	2 (4%)
Alveolar epithelium, hyperplasia	8 (16%)	4 (8%)	2 (4%)	5 (10%)
Bronchiole, hyperplasia	1 (2%)			
Bronchus, hyperplasia	1 (2%)			
Nose	(50)	(49)	(49)	(50)
Foreign body	11 (22%)	5 (10%)	9 (18%)	5 (10%)
Hemorrhage			1 (2%)	
Inflammation, chronic active	33 (66%)	32 (65%)	38 (78%)	46 (92%)
Respiratory metaplasia	1 (2%)			
Synechia, focal	1 (2%)		1 (2%)	
Thrombosis			1 (2%)	
Glands, hyperplasia	1 (2%)			
Goblet cell, respiratory epithelium, hyperplasia	20 (40%)	18 (37%)	41 (84%)	34 (68%)
Goblet cell, transitional epithelium, hyperplasia			1 (2%)	
Nasolacrimal duct, inflammation, chronic active	1 (2%)			
Nerve, olfactory epithelium, atrophy		17 (35%)	14 (29%)	23 (46%)
Olfactory epithelium, accumulation, hyaline droplet	45 (90%)	43 (88%)	14 (29%)	
Olfactory epithelium, atrophy	1 (2%)	26 (53%)	37 (76%)	31 (62%)
Olfactory epithelium, foreign body			1 (2%)	
Olfactory epithelium, hyperplasia				1 (2%)
Olfactory epithelium, pigmentation		39 (80%)	42 (86%)	30 (60%)
Olfactory epithelium, respiratory metaplasia	9 (18%)	30 (61%)	40 (82%)	32 (64%)
Respiratory epithelium, hyperplasia	14 (28%)	28 (57%)	45 (92%)	35 (70%)
Respiratory epithelium, inflammation, chronic	1 (2%)			
Submucosa, fibrosis				8 (16%)
Transitional epithelium, hyperplasia	2 (4%)	18 (37%)	43 (88%)	31 (62%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Respiratory System (continued)				
Pleura	(0)	(0)	(1)	(0)
Trachea	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)			2 (4%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	1 (2%)	4 (8%)		
Degeneration		1 (2%)		
Anterior chamber, inflammation, suppurative				2 (4%)
Retina, atrophy		1 (2%)		
Harderian gland	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Inflammation		2 (4%)		
Zymbal's gland	(1)	(0)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hydropnephrosis	1 (2%)			1 (2%)
Infarct			1 (2%)	
Nephropathy	49 (98%)	49 (98%)	49 (98%)	50 (100%)
Papilla, necrosis	3 (6%)	1 (2%)		1 (2%)
Pelvis, inflammation, acute				1 (2%)
Renal tubule, cyst	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Renal tubule, hyperplasia	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation, acute				2 (4%)
Inflammation, chronic	2 (4%)	1 (2%)		2 (4%)
Mineralization				1 (2%)
Necrosis	1 (2%)			
Ulcer				2 (4%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF *GINKGO BILOBA* EXTRACT

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	9	13	7	4
Natural deaths	4	10	6	13
Survivors				
Died last week of study			1	
Terminal kill	37	27	36	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uterus			1 (2%)	
Intestine large, cecum	(50)	(50)	(50)	(49)
Intestine large, colon	(50)	(50)	(50)	(49)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(49)
Schwannoma, malignant	1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(49)
Carcinoma, metastatic, uterus			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(49)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uterus			1 (2%)	
Mesentery	(10)	(2)	(8)	(11)
Oral mucosa	(0)	(0)	(1)	(0)
Squamous cell papilloma			1 (100%)	
Pancreas	(50)	(50)	(50)	(48)
Carcinoma, metastatic, uterus			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma		1 (2%)		
Carcinoma, metastatic, uterus			1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	2 (4%)	2 (4%)		2 (4%)
Pheochromocytoma malignant				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			1 (2%)
Parathyroid gland	(38)	(47)	(43)	(35)
Adenoma	1 (3%)			
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	33 (66%)	27 (54%)	22 (44%)	16 (32%)
Pars distalis, adenoma, multiple	1 (2%)	1 (2%)		
Pars distalis, carcinoma	2 (4%)			2 (4%)
Pars intermedia, adenoma	1 (2%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Endocrine system (continued)				
Thyroid gland	(49)	(50)	(49)	(49)
Bilateral, C-cell, adenoma	1 (2%)			
C-cell, adenoma	10 (20%)	8 (16%)	8 (16%)	5 (10%)
C-cell, carcinoma		1 (2%)	1 (2%)	1 (2%)
Follicle, adenoma	1 (2%)		3 (6%)	1 (2%)
Follicle, carcinoma			1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	5 (10%)	5 (10%)	4 (8%)	7 (14%)
Carcinoma			1 (2%)	
Bilateral, adenoma		3 (6%)		1 (2%)
Ovary	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uterus			1 (2%)	
Granulosa cell tumor benign		1 (2%)	1 (2%)	
Schwannoma malignant, metastatic, intestine small, duodenum	1 (2%)			
Tubulostromal adenoma	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Polyp stromal	3 (6%)	8 (16%)	8 (16%)	9 (18%)
Schwannoma malignant	1 (2%)			
Endometrium adenoma				1 (2%)
Vagina	(0)	(2)	(0)	(0)
Schwannoma malignant		2 (100%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(3)	(3)	(3)	(4)
Mediastinal, carcinoma, metastatic, thyroid gland		1 (33%)		
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uterus			1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Thymus	(50)	(48)	(49)	(48)
Thymoma benign	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibroadenoma	13 (26%)	22 (44%)	21 (42%)	10 (20%)
Fibroadenoma, multiple	10 (20%)	9 (18%)	10 (20%)	4 (8%)
Skin	(50)	(50)	(50)	(50)
Keratoacanthoma				1 (2%)
Squamous cell carcinoma	1 (2%)			
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Subcutaneous tissue, fibrosarcoma			1 (2%)	1 (2%)
Subcutaneous tissue, schwannoma malignant		1 (2%)	1 (2%)	1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(0)	(1)	(0)
Carcinoma, metastatic, uterus			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant		1 (2%)		
Granular cell tumor malignant				1 (2%)
Peripheral nerve	(1)	(0)	(0)	(0)
Spinal cord	(1)	(0)	(0)	(0)
Respiratory System				
Larynx	(0)	(0)	(0)	(1)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)		3 (6%)	
Astrocytoma malignant, metastatic, brain		1 (2%)		
Carcinoma, metastatic, adrenal cortex		1 (2%)		
Carcinoma, metastatic, uterus			1 (2%)	
Granular cell tumor malignant, metastatic, brain				1 (2%)
Nose	(49)	(49)	(50)	(46)
Respiratory epithelium, adenoma			2 (4%)	
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uterus			1 (2%)	
Mesenchymal tumor malignant				1 (2%)
Renal tubule, adenoma				1 (2%)
Urinary bladder	(50)	(50)	(50)	(49)
Carcinoma, metastatic, uterus			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Leukemia mononuclear	8 (16%)	8 (16%)	7 (14%)	5 (10%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	47	48	42
Total primary neoplasms	102	101	99	75
Total animals with benign neoplasms	47	43	46	37
Total benign neoplasms	89	87	86	60
Total animals with malignant neoplasms	12	14	12	13
Total malignant neoplasms	13	14	13	15
Total animals with metastatic neoplasms	1	3	1	1
Total metastatic neoplasms	1	3	11	1

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

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate[a]	2/50 (4%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate[b]	4.4%	4.9%	0.0%	7.2%
Terminal rate[c]	1/37 (3%)	2/27 (7%)	0/37 (0%)	2/32 (6%)
First incidence (days)	653	728 (T)	—[e]	618
Poly-3 test[d]	P=0.329	P=0.652	P=0.237N	P=0.458
Clitoral Gland: Adenoma				
Overall rate	5/50 (10%)	8/50 (16%)	4/50 (8%)	8/50 (16%)
Adjusted rate	11.0%	19.2%	8.8%	19.0%
Terminal rate	4/37 (11%)	7/27 (26%)	4/37 (11%)	4/32 (13%)
First incidence (days)	698	380	728 (T)	587
Poly-3 test	P=0.269	P=0.218	P=0.498N	P=0.226
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	8/50 (16%)	5/50 (10%)	8/50 (16%)
Adjusted rate	11.1%	19.2%	10.8%	19.0%
Terminal rate	4/37 (11%)	7/27 (14%)	4/37 (11%)	4/32 (13%)
First incidence (days)	698	380	533	587
Poly-3 test	P=0.279	P=0.218	P=0.620N	P=0.226
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	8.8%	0.0%	6.6%	0.0%
Terminal rate	4/37 (11%)	0/27 (0%)	2/37 (5%)	0/32 (0%)
First incidence (days)	728 (T)	—	710	—
Poly-3 test	P=0.117N	P=0.075N	P=0.496N	P=0.073N
Mammary Gland: Fibroadenoma				
Overall rate	23/50 (46%)	31/50 (62%)	31/50 (62%)	14/50 (28%)
Adjusted rate	49.7%	70.4%	64.8%	33.4%
Terminal rate	20/37 (54%)	19/27 (70%)	24/37 (65%)	10/32 (31%)
First incidence (days)	596	559	474	639
Poly-3 test	P=0.004N	P=0.031	P=0.097	P=0.086N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	34/50 (68%)	28/50 (56%)	22/50 (44%)	16/50 (32%)
Adjusted rate	70.7%	62.2%	47.0%	37.8%
Terminal rate	24/37 (65%)	16/27 (59%)	17/37 (46%)	13/32 (41%)
First incidence (days)	551	433	609	533
Poly-3 test	P<0.001N	P=0.255N	P=0.013N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	36/50 (72%)	28/50 (56%)	22/50 (44%)	18/50 (36%)
Adjusted rate	74.1%	62.2%	47.0%	42.6%
Terminal rate	25/37 (68%)	16/27 (59%)	17/37 (46%)	14/32 (44%)
First incidence (days)	551	433	609	533
Poly-3 test	P=0.002N	P=0.149N	P=0.004N	P<0.001N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.2%	2.5%	6.5%	4.8%
Terminal rate	1/37 (3%)	1/27 (4%)	2/37 (5%)	1/32 (3%)
First incidence (days)	728 (T)	728 (T)	656	533
Poly-3 test	P=0.401	P=0.736	P=0.309	P=0.472

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Thyroid Gland (C-Cell): Adenoma				
Overall rate	11/49 (22%)	8/50 (16%)	8/49 (16%)	5/49 (10%)
Adjusted rate	24.0%	19.6%	17.5%	12.1%
Terminal rate	7/37 (19%)	7/27 (26%)	6/37 (16%)	3/32 (9%)
First incidence (days)	498	705	656	618
Poly-3 test	P=0.116N	P=0.408N	P=0.307N	P=0.124N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	11/49 (22%)	9/50 (18%)	9/49 (18%)	6/49 (12%)
Adjusted rate	24.0%	22.0%	19.7%	14.4%
Terminal rate	7/37 (19%)	7/27 (26%)	7/37 (19%)	3/32 (9%)
First incidence (days)	498	692	656	618
Poly-3 test	P=0.166N	P=0.513N	P=0.404N	P=0.196N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	1/49 (2%)	0/50 (0%)	3/49 (6%)	1/49 (2%)
Adjusted rate	2.2%	0.0%	6.6%	2.4%
Terminal rate	1/37 (3%)	0/27 (0%)	3/37 (8%)	0/32 (0%)
First incidence (days)	728 (T)	—	728 (T)	637
Poly-3 test	P=0.573	P=0.519N	P=0.310	P=0.741
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/49 (2%)	0/50 (0%)	4/49 (8%)	2/49 (4%)
Adjusted rate	2.2%	0.0%	8.8%	4.9%
Terminal rate	1/37 (3%)	0/27 (0%)	4/37 (11%)	1/32 (3%)
First incidence (days)	728 (T)	—	728 (T)	637
Poly-3 test	P=0.324	P=0.519N	P=0.183	P=0.469
Uterus: Stromal Polyp				
Overall rate	3/50 (6%)	8/50 (16%)	8/50 (16%)	9/50 (18%)
Adjusted rate	6.6%	18.8%	17.3%	20.7%
Terminal rate	2/37 (5%)	4/27 (15%)	7/27 (19%)	4/32 (13%)
First incidence (days)	653	433	533	521
Poly-3 test	P=0.120	P=0.077	P=0.102	P=0.048
All Organs: Mononuclear Cell Leukemia				
Overall rate	8/50 (16%)	8/50 (16%)	7/50 (14%)	5/50 (10%)
Adjusted rate	17.5%	19.1%	14.9%	12.1%
Terminal rate	6/37 (16%)	4/27 (15%)	4/37 (11%)	5/32 (16%)
First incidence (days)	653	629	580	728 (T)
Poly-3 test	P=0.258N	P=0.530	P=0.480N	P=0.344N
All Organs: Benign Neoplasms				
Overall rate	47/50 (94%)	43/50 (86%)	46/50 (92%)	37/50 (74%)
Adjusted rate	95.5%	90.5%	93.1%	81.2%
Terminal rate	35/37 (95%)	25/27 (93%)	35/37 (95%)	25/32 (78%)
First incidence (days)	498	380	474	521
Poly-3 test	P=0.014N	P=0.279N	P=0.474N	P=0.025N
All Organs: Malignant Neoplasms				
Overall rate	12/50 (24%)	14/50 (28%)	12/50 (24%)	13/50 (26%)
Adjusted rate	25.4%	32.0%	25.1%	30.4%
Terminal rate	7/37 (19%)	5/27 (19%)	6/37 (16%)	9/32 (28%)
First incidence (days)	454	327	533	533
Poly-3 test	P=0.430	P=0.320	P=0.580N	P=0.383

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	47/50 (94%)	48/50 (96%)	42/50 (84%)
Adjusted rate	98.0%	95.6%	96.0%	91.1%
Terminal rate	36/37 (97%)	25/27 (93%)	35/37 (100%)	29/32 (91%)
First incidence (days)	454	327	474	521
Poly-3 test	P=0.100N	P=0.460N	P=0.500N	P=0.137N

(T) Terminal kill

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

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value 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 Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

TABLE B3a
Historical Incidence of Neoplasms of the Thyroid Gland in Control Female F344/N Rats[a]

Study (Study Start)	Follicular Cell Adenoma	Follicular Cell Carcinoma	Follicular Cell Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
Ginkgo biloba extract (March 2005)	1/49	0/49	1/49
Isoeugenol (April 2002)	0/50	1/50	1/50
Kava kava extract (August 2004)	1/50	0/50	1/50
β-Myrcene (March 2002)	0/50	0/50	0/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	1/49	0/49	1/49
Pulegone (April 2003)	0/50	0/50	0/50
Total (%)	3/298 (1.0%)	1/298 (0.3%)	4/298 (1.3%)
Mean ± standard deviation	1.0% ± 1.1%	0.3% ± 0.8%	1.4% ± 1.0%
Range	0%-2%	0%-2%	0%-2%
Overall Historical Incidence: All Routes			
Total (%)	8/1,186 (0.7%)	5/1,186 (0.4%)	12/1,186 (1.0%)
Mean ± standard deviation	0.7% ± 1.0%	0.4% ± 1.0%	1.0% ± 1.3%
Range	0%-2%	0%-4%	0%-4%

[a] Data as of May 2011

TABLE B3b
Historical Incidence of Adenoma of the Nose in Control Female F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
Ginkgo biloba extract (March 2005)	0/49
Isoeugenol (April 2002)	0/50
Kava kava extract (August 2004)	0/50
β-Myrcene (March 2002)	0/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	0/50
Pulegone (April 2003)	0/50
Total	0/299
Overall Historical Incidence: All Routes	
Total (%)	1/1,196 (0.1%)
Mean ± standard deviation	0.1% ± 0.4%
Range	0%-2%

[a] Data as of May 2011

TABLE B3c
Historical Incidence of Stromal Polyp of the Uterus in Control Female F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
Ginkgo biloba extract (March 2005)	3/50
Isoeugenol (April 2002)	8/50
Kava kava extract (August 2004)	7/50
β-Myrcene (March 2002)	8/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	3/50
Pulegone (April 2003)	5/50
Total (%)	34/300 (11.3%)
Mean ± standard deviation	11.3% ± 4.7%
Range	6%-16%
Overall Historical Incidence: All Routes	
Total (%)	189/1,200 (15.8%)
Mean ± standard deviation	15.8% ± 6.6%
Range	4%-34%

[a] Data as of May 2011

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	9	13	7	4
Natural deaths	4	10	6	13
Survivors				
Died last week of study			1	
Terminal kill	37	27	36	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(49)
Inflammation, chronic			2 (4%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan	3 (6%)	2 (4%)	2 (4%)	
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	8 (16%)	4 (8%)	3 (6%)	6 (12%)
Intestine small, duodenum	(50)	(50)	(50)	(49)
Intestine small, ileum	(50)	(50)	(50)	(49)
Intestine small, jejunum	(50)	(50)	(50)	(49)
Liver	(50)	(50)	(50)	(50)
Angiectasis	7 (14%)	2 (4%)	4 (8%)	4 (8%)
Basophilic focus	48 (96%)	43 (86%)	44 (88%)	40 (80%)
Clear cell focus	20 (40%)	7 (14%)	17 (34%)	11 (22%)
Degeneration, cystic	1 (2%)		2 (4%)	
Eosinophilic focus	24 (48%)	30 (60%)	38 (76%)	30 (60%)
Fatty change, focal	11 (22%)	25 (50%)	30 (60%)	25 (50%)
Hepatodiaphragmatic nodule	6 (12%)	7 (14%)	1 (2%)	3 (6%)
Inflammation, chronic	45 (90%)	41 (82%)	41 (82%)	42 (84%)
Mixed cell focus	24 (48%)	12 (24%)	17 (34%)	10 (20%)
Necrosis	5 (10%)	2 (4%)	3 (6%)	3 (6%)
Bile duct, hyperplasia	11 (22%)	31 (62%)	31 (62%)	33 (66%)
Hepatocyte, fatty change	14 (28%)	7 (14%)	7 (14%)	9 (18%)
Hepatocyte, hypertrophy	7 (14%)	15 (30%)	27 (54%)	33 (66%)
Ito cell, hyperplasia		1 (2%)		
Mesentery	(10)	(2)	(8)	(11)
Fat, necrosis	10 (100%)	2 (100%)	8 (100%)	11 (100%)
Oral mucosa	(0)	(0)	(1)	(0)
Pancreas	(50)	(50)	(50)	(48)
Hemorrhage				1 (2%)
Acinus, atrophy	17 (34%)	13 (26%)	12 (24%)	20 (42%)
Acinus, hyperplasia	5 (10%)		2 (4%)	
Duct, cyst	3 (6%)		1 (2%)	4 (8%)
Duct, hyperplasia		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Ulcer	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation, chronic		2 (4%)		
Mineralization	1 (2%)	2 (4%)		1 (2%)
Ulcer		2 (4%)	3 (6%)	
Glands, ectasia	35 (70%)	37 (74%)	37 (74%)	36 (72%)
Glands, hyperplasia	1 (2%)		1 (2%)	

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, mineralization		1 (2%)		
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	49 (98%)	46 (92%)	46 (92%)	48 (96%)
Atrium, thrombosis		1 (2%)		1 (2%)
Myocardium, mineralization				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Degeneration, cystic	12 (24%)	5 (10%)	7 (14%)	7 (14%)
Hyperplasia, focal	12 (24%)	10 (20%)	13 (26%)	15 (30%)
Hyperplasia, diffuse				1 (2%)
Hypertrophy, focal	6 (12%)	2 (4%)	2 (4%)	5 (10%)
Hypertrophy, diffuse	3 (6%)	10 (20%)	7 (14%)	9 (18%)
Vacuolization cytoplasmic, focal	23 (46%)	14 (28%)	22 (44%)	21 (42%)
Vacuolization cytoplasmic, diffuse	4 (8%)	5 (10%)	6 (12%)	4 (8%)
Capsule, fibrosis	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	3 (6%))	5 (10%)	2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			
Parathyroid gland	(38)	(47)	(43)	(35)
Hyperplasia	1 (3%)			
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis		2 (4%)		
Cyst	26 (52%)	24 (48%)	29 (58%)	31 (62%)
Pars distalis, cyst	1 (2%)			
Pars distalis, hyperplasia	11 (22%)	13 (26%)	18 (36%)	23 (46%)
Thyroid gland	(49)	(50)	(49)	(49)
C-cell, hyperplasia	40 (82%)	38 (76%)	36 (73%)	21 (43%)
Follicle, hyperplasia	3 (6%)	3 (6%)	1 (2%)	5 (10%)
Follicular cell, hypertrophy	15 (31%)	41 (82%)	45 (92%)	48 (98%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Cyst			1 (2%)	
Hyperplasia	3 (6%)	4 (8%)	3 (6%)	2 (4%)
Inflammation, chronic	36 (72%)	39 (78%)	40 (80%)	38 (76%)
Mineralization		1 (2%)		
Ovary	(50)	(50)	(50)	(50)
Cyst	3 (6%)	2 (4%)	2 (4%)	3 (6%)
Uterus	(50)	(50)	(50)	(50)
Cyst	4 (8%)			4 (8%)
Decidual reaction			1 (2%)	
Inflammation, suppurative	2 (4%)	2 (4%)		5 (10%)
Necrosis		1 (2%)		
Endometrium, hyperplasia, cystic	3 (6%)	13 (26%)	11 (22%)	7 (14%)
Vagina	(0)	(2)	(0)	(0)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Hyperplasia	7 (14%)	11 (22%)	9 (18%)	12 (24%)
Necrosis			1 (2%)	
Lymph node	(3)	(3)	(3)	(4)
Ectasia				1 (25%)
Deep cervical, hyperplasia, lymphoid		1 (33%)		
Mediastinal, ectasia				1 (25%)
Mediastinal, hemorrhage		1 (33%)		
Mediastinal, hyperplasia, lymphoid			1 (33%)	3 (75%)
Pancreatic, congestion	1 (33%)			
Lymph node, mesenteric	(50)	(50)	(50)	(49)
Pigmentation, hemosiderin				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	14 (28%)	18 (36%)	13 (26%)	13 (26%)
Hyperplasia, histiocytic	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Infarct		1 (2%)		
Necrosis			1 (2%)	
Thrombosis		1 (2%)		
Lymphoid follicle, atrophy	9 (18%)	6 (12%)	9 (18%)	5 (10%)
Thymus	(50)	(48)	(49)	(48)
Atrophy	48 (96%)	47 (98%)	46 (94%)	47 (98%)
Ectopic parathyroid gland		1 (2%)		
Ectopic thyroid		1 (2%)		1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Duct, cyst	14 (28%)	10 (20%)	8 (16%)	6 (12%)
Skin	(50)	(50)	(50)	(50)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis				1 (2%)
Skeletal muscle	(0)	(0)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Congestion				1 (2%)
Hydrocephalus	1 (2%)			
Artery, meninges, inflammation, chronic		1 (2%)		
Hippocampus, necrosis				1 (2%)
Hypothalamus, congestion		1 (2%)		
Peripheral nerve	(1)	(0)	(0)	(0)
Spinal cord	(1)	(0)	(0)	(0)
Congestion	1 (100%)			
Respiratory System				
Larynx	(0)	(0)	(0)	(1)
Foreign body				1 (100%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Respiratory System (continued)				
Lung	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Inflammation, suppurative			1 (2%)	
Inflammation, chronic	7 (14%)	3 (6%)	11 (22%)	6 (12%)
Inflammation, chronic active				1 (2%)
Metaplasia, osseous	2 (4%)	1 (2%)		1 (2%)
Thrombosis			1 (2%)	
Alveolar epithelium, hyperplasia	3 (6%)		3 (6%)	2 (4%)
Nose	(49)	(49)	(50)	(46)
Foreign body	1 (2%)	1 (2%)	1 (2%)	7 (15%)
Inflammation, chronic active	22 (45%)	16 (33%)	26 (52%)	38 (83%)
Goblet cell, olfactory epithelium, hyperplasia		1 (2%)		
Goblet cell, respiratory epithelium, hyperplasia	6 (12%)	2 (4%)	18 (36%)	35 (76%)
Nerve, olfactory epithelium, atrophy		15 (31%)	22 (44%)	33 (72%)
Olfactory epithelium, accumulation, hyaline droplet	44 (90%)	39 (80%)	25 (50%)	
Olfactory epithelium, atrophy		18 (37%)	25 (50%)	37 (80%)
Olfactory epithelium, degeneration		1 (2%)		
Olfactory epithelium, hemorrhage	1 (2%)			
Olfactory epithelium, hyperplasia				1 (2%)
Olfactory epithelium, pigmentation		37 (76%)	43 (86%)	40 (87%)
Olfactory epithelium, regeneration			1 (2%)	2 (4%)
Olfactory epithelium, respiratory metaplasia	8 (16%)	4 (8%)	32 (64%)	37 (80%)
Respiratory epithelium, hyperplasia	9 (18%)	9 (18%)	19 (38%)	34 (74%)
Transitional epithelium, hyperplasia		6 (12%)	32 (64%)	36 (78%)
Trachea	(50)	(50)	(50)	(50)
Inflammation, chronic			1 (2%)	1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract		2 (4%)	3 (6%)	2 (4%)
Degeneration			1 (2%)	
Retinal detachment				1 (2%)
Retina, atrophy		2 (4%)	1 (2%)	
Retina, hemorrhage				1 (2%)
Retrobulbar, inflammation, chronic			1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Inflammation, chronic	4 (8%)	6 (12%)	8 (16%)	7 (14%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hydropnephrosis			1 (2%)	
Infarct	2 (4%)	2 (4%)	1 (2%)	6 (12%)
Inflammation, chronic	2 (4%)		1 (2%)	
Nephropathy	41 (82%)	42 (84%)	43 (86%)	42 (84%)
Renal tubule, cyst			1 (2%)	
Renal tubule, hyperplasia	3 (6%)	1 (2%)	3 (6%)	
Renal tubule, mineralization	1 (2%)	1 (2%)		1 (2%)
Renal tubule, pigmentation, lipofuscin	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation, acute	1 (2%)			

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF *GINKGO BILOBA* EXTRACT

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death			1	
Moribund	8	16	23	13
Natural deaths	8	7	5	14
Survivors				
Terminal kill	34	27	21	23
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(46)	(48)	(44)	(46)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)		1 (2%)	2 (4%)
Serosa, hepatoblastoma		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	9 (18%)	2 (4%)		3 (6%)
Hemangiosarcoma, multiple		1 (2%)		
Hepatoblastoma	2 (4%)	16 (32%)	10 (20%)	17 (34%)
Hepatoblastoma, metastatic, liver				1 (2%)
Hepatoblastoma, multiple	1 (2%)	12 (24%)	26 (52%)	21 (42%)
Hepatocellular adenoma	13 (26%)	6 (12%)	7 (14%)	6 (12%)
Hepatocellular adenoma, multiple	18 (36%)	40 (80%)	26 (52%)	27 (54%)
Hepatocellular carcinoma	11 (22%)	8 (16%)	9 (18%)	4 (8%)
Hepatocellular carcinoma, multiple	11 (22%)	23 (46%)	32 (64%)	43 (86%)
Hepatocholangiocarcinoma		1 (2%)		
Hepatocholangiocarcinoma, multiple		1 (2%)		1 (2%)
Ito cell tumor benign		1 (2%)		
Ito cell tumor benign, multiple	1 (2%)			
Mesentery	(5)	(6)	(3)	(4)
Hepatoblastoma, metastatic, liver		2 (33%)	1 (33%)	1 (25%)
Hepatocholangiocarcinoma, metastatic, liver				1 (25%)
Pancreas	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver		1 (2%)	1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma		2 (4%)	1 (2%)	
Squamous cell papilloma, multiple				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(50)	(50)	(50)	(50)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, liver	1 (2%)	1 (2%)		
Hepatoblastoma, metastatic, liver		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver				1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver			1 (2%)	
Subcapsular, adenoma	1 (2%)	3 (6%)	1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(49)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Parathyroid gland	(36)	(46)	(46)	(44)
Pituitary gland	(49)	(50)	(50)	(49)
Thyroid gland	(49)	(49)	(50)	(50)
C-cell, carcinoma			1 (2%)	1 (2%)
Follicular cell, adenoma			2 (4%)	2 (4%)
General Body System				
Peritoneum	(0)	(1)	(2)	(2)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver		1 (2%)		
Bilateral, hepatoblastoma, metastatic, liver				1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Prostate	(50)	(50)	(49)	(50)
Adenoma				1 (2%)
Carcinoma		1 (2%)		
Hemangiosarcoma		1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	1 (2%)		1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangioma			1 (2%)	
Lymph node	(11)	(15)	(18)	(14)
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung		1 (7%)		
Bronchial, hepatoblastoma, metastatic, liver		1 (7%)		
Bronchial, hepatocholangiocarcinoma, metastatic, liver				1 (7%)
Lymph node, mandibular	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Harderian gland			1 (2%)	
Lymph node, mesenteric	(50)	(49)	(49)	(50)
Hepatoblastoma, metastatic, liver			1 (2%)	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		1 (2%)
Thymus	(50)	(47)	(50)	(45)
Thymoma malignant		1 (2%)		
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Pinna, amelanotic melanoma, malignant				1 (2%)
Pinna, melanoma malignant	1 (2%)			
Tail, schwannoma NOS				1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Turbinate, carcinoma, metastatic, Harderian gland			1 (2%)	
Skeletal muscle	(1)	(1)	(1)	(1)
Hepatoblastoma, metastatic, liver		1 (100%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Neuroblastoma			1 (2%)	
Peripheral nerve	(0)	(0)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	8 (16%)	6 (12%)	5 (10%)	1 (2%)
Alveolar/bronchiolar carcinoma	11 (22%)	7 (14%)	4 (8%)	3 (6%)
Alveolar/bronchiolar carcinoma, metastatic, lung	2 (4%)			
Alveolar/bronchiolar carcinoma, multiple		1 (2%)	1 (2%)	
Carcinoma, metastatic, Harderian gland			1 (2%)	
Hemangiosarcoma	1 (2%)			
Hepatoblastoma, metastatic, liver		7 (14%)	14 (28%)	14 (28%)
Hepatocellular carcinoma, metastatic, liver	10 (20%)	8 (16%)	8 (16%)	18 (36%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		1 (2%)
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(1)	(0)	(0)	(1)
Neural crest tumor				1 (100%)
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	3 (6%)	2 (4%)	3 (6%)
Carcinoma	3 (6%)	5 (10%)	4 (8%)	2 (4%)
Hepatoblastoma, metastatic, liver			1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver		1 (2%)		1 (2%)
Urethra	(0)	(1)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Lymphoma malignant	1 (2%)	3 (6%)	1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	44	48	48	49
Total primary neoplasms	99	148	137	144
Total animals with benign neoplasms	38	47	36	35
Total benign neoplasms	47	61	46	42
Total animals with malignant neoplasms	35	46	46	48
Total malignant neoplasms	52	87	91	100
Total animals with metastatic neoplasms	13	17	23	26
Total metastatic neoplasms	13	26	31	42
Total animals with uncertain neoplasms benign or malignant				2
Total uncertain neoplasms				2

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

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	1/50 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate[b]	2.3%	7.3%	2.6%	0.0%
Terminal rate[c]	0/34 (0%)	2/27 (7%)	0/21 (0%)	0/23 (0%)
First incidence (days)	660	565	681	—[e]
Poly-3 test[d]	P=0.196N	P=0.277	P=0.727	P=.519N
Harderian Gland: Adenoma				
Overall rate	4/50 (8%)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rate	9.1%	7.4%	5.1%	7.4%
Terminal rate	4/34 (12%)	3/27 (11%)	1/21 (5%)	2/23 (9%)
First incidence (days)	726 (T)	726 (T)	308	641
Poly-3 test	P=0.532N	P=0.547N	P=0.394N	P=545N
Harderian Gland: Carcinoma				
Overall rate	3/50 (6%)	5/50 (10%)	4/50 (8%)	2/50 (4%)
Adjusted rate	6.8%	11.9%	10.1%	4.9%
Terminal rate	3/34 (9%)	1/27 (4%)	0/21 (0%)	1/23 (4%)
First incidence (days)	726 (T)	520	490	620
Poly-3 test	P=0.309N	P=0.333	P=0.446	P=537N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	8/50 (16%)	6/50 (12%)	5/50 (10%)
Adjusted rate	15.9%	19.0%	14.7%	12.2%
Terminal rate	7/34 (21%)	4/27 (15%)	1/21 (5%)	3/23 (13%)
First incidence (days)	726 (T)	520	308	620
Poly-3 test	P=0.305N	P=0.464	P=0.559N	P=0.431N
Liver: Hemangiosarcoma				
Overall rate	9/50 (18%)	3/50 (6%)	0/50 (0%)	3/50 (6%)
Adjusted rate	20.1%	7.4%	0.0%	7.4%
Terminal rate	6/34 (18%)	1/27 (4%)	0/21 (0%)	2/23 (9%)
First incidence (days)	594	674	—	641
Poly-3 test	P=0.126N	P=0.083N	P=0.004N	P=0.083N
Liver: Hepatocellular Adenoma				
Overall rate	31/50 (62%)	46/50 (92%)	33/50 (66%)	33/50 (66%)
Adjusted rate	66.2%	96.0%	74.3%	71.0%
Terminal rate	22/34 (65%)	27/27 (100%)	16/21 (76%)	16/23 (70%)
First incidence (days)	482	400	308	553
Poly-3 test	P=0.171N	P<0.001	P=0.260	P=0.388
Liver: Hepatocellular Carcinoma				
Overall rate	22/50 (44%)	31/50 (62%)	41/50 (82%)	47/50 (94%)
Adjusted rate	47.0%	70.6%	92.5%	98.8%
Terminal rate	13/34 (38%)	20/27 (74%)	20/21 (95%)	23/23 (100%)
First incidence (days)	442	555	490	553
Poly-3 test	P<0.001	P=0.015	P<0.001	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	39/50 (78%)	46/50 (92%)	46/50 (92%)	49/50 (98%)
Adjusted rate	79.4%	96.0%	98.5%	100.0%
Terminal rate	25/34 (74%)	27/27 (100%)	21/21 (100%)	23/23 (100%)
First incidence (days)	442	400	308	443
Poly-3 test	P<0.001	P=0.011	P=0.002	P<0.001

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Liver: Hepatoblastoma				
Overall rate	3/50 (6%)	28/50 (56%)	36/50 (72%)	38/50 (76%)
Adjusted rate	6.8%	61.0%	80.6%	79.6%
Terminal rate	3/34 (9%)	13/27 (48%)	14/21 (67%)	16/23 (70%)
First incidence (days)	726 (T)	400	477	516
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	24/50 (48%)	42/50 (84%)	45/50 (90%)	48/50 (96%)
Adjusted rate	51.3%	88.3%	97.1%	99.5%
Terminal rate	15/34 (44%)	23/27 (85%)	20/21 (95%)	23/23 (100%)
First incidence (days)	442	400	477	516
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	39/50 (78%)	48/50 (96%)	48/50 (96%)	49/50 (98%)
Adjusted rate	79.4%	98.5%	100.0%	100.0%
Terminal rate	25/34 (74%)	27/27 (100%)	21/21 (100%)	23/23 (100%)
First incidence (days)	442	400	308	443
Poly-3 test	P<0.001	P=0.002	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	8/50 (16%)	6/50 (12%)	5/50 (10%)	1/50 (2%)
Adjusted rate	17.9%	14.4%	13.0%	2.5%
Terminal rate	5/34 (15%)	2/27 (7%)	3/21 (14%)	1/23 (4%)
First incidence (days)	592	555	611	726 (T)
Poly-3 test	P=0.020N	P=0.444N	P=0.377N	P=0.024N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	11/50 (22%)	8/50 (16%)	5/50 (10%)	3/50 (6%)
Adjusted rate	24.4%	19.7%	13.2%	7.3%
Terminal rate	8/34 (24%)	7/27 (26%)	5/21 (24%)	0/23 (0%)
First incidence (days)	482	674	726 (T)	553
Poly-3 test	P=0.022N	P=0.399N	P=0.155N	P=0.029N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	17/50 (34%)	14/50 (28%)	10/50 (20%)	4/50 (8%)
Adjusted rate	37.1%	33.5%	25.9%	9.7%
Terminal rate	12/34 (35%)	9/27 (33%)	8/21 (38%)	1/23 (4%)
First incidence (days)	482	555	611	553
Poly-3 test	P<0.001N	P=0.450N	P=0.193N	P=0.002N
All Organs: Hemangiosarcoma				
Overall rate	9/50 (18%)	5/50 (10%)	0/50 (0%)	4/50 (8%)
Adjusted rate	20.1%	12.3%	0.0%	9.9%
Terminal rate	6/34 (18%)	3/27 (11%)	0/21 (0%)	3/23 (13%)
First incidence (days)	594	674	—	641
Poly-3 test	P=0.178N	P=0.249N	P=0.004N	P=0.156N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	9/50 (18%)	5/50 (10%)	1/50 (2%)	4/50 (8%)
Adjusted rate	20.1%	12.3%	2.6%	9.9%
Terminal rate	6/34 (18%)	3/27 (11%)	1/21 (5%)	3/23 (13%)
First incidence (days)	594	674	726 (T)	641
Poly-3 test	P=0.180N	P=0.249N	P=0.016N	P=0.156N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
All Organs: Malignant Lymphoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.3%	7.4%	2.6%	0.0%
Terminal rate	1/34 (3%)	2/27 (7%)	1/21 (5%)	0/23 (0%)
First incidence (days)	726 (T)	687	726 (T)	—
Poly-3 test	P=0.195N	P=0.276	P=0.727	P=0.518N
All Organs: Benign Neoplasms				
Overall rate	38/50 (76%)	47/50 (94%)	36/50 (72%)	35/50 (70%)
Adjusted rate	79.8%	97.7%	80.3%	75.2%
Terminal rate	26/34 (77%)	27/27 (100%)	18/21 (86%)	17/23 (74%)
First incidence (days)	482	400	308	443
Poly-3 test	P=0.040N	P=0.003	P=0.581	P=0.383N
All Organs: Malignant Neoplasms				
Overall rate	35/50 (70%)	46/50 (92%)	46/50 (92%)	48/50 (96%)
Adjusted rate	71.9%	94.4%	97.7%	99.5%
Terminal rate	22/34 (65%)	25/27 (93%)	20/21 (95%)	23/23 (100%)
First incidence (days)	442	400	453	516
Poly-3 test	P<0.001	P=0.002	P<0.001	P<0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	44/50 (88%)	48/50 (96%)	48/50 (96%)	49/50 (98%)
Adjusted rate	89.5%	98.5%	100.0%	100.0%
Terminal rate	29/34 (85%)	27/27 (100%)	21/21 (100%)	23/23 (100%)
First incidence (days)	442	400	308	443
Poly-3 test	P=0.030	P=0.066	P=0.028	P=0.026

(T) Terminal kill

[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] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value 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 Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

TABLE C3a
Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
Ginkgo biloba extract (March 2005)	31/50	22/50	39/50
Isoeugenol (April 2002)	24/50	8/50	28/50
Kava kava extract (August 2004)	27/50	20/50	38/50
β-Myrcene (March 2002)	26/50	14/50	33/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	29/50	22/50	38/50
Pulegone (April 2003)	22/50	13/50	29/50
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	22/50	17/50	34/50
Total (%)	181/350 (51.7%)	116/350 (33.1%)	239/350 (68.3%)
Mean ± standard deviation	51.7% ± 6.9%	33.1% ± 10.5%	68.3% ± 8.9%
Range	44%-62%	16%-44%	56%-78%
Overall Historical Incidence: All Routes			
Total (%)	658/1,149 (57.3%)	399/1,149 (34.7%)	844/1,149 (73.5%)
Mean ± standard deviation	57.3% ± 12.6%	34.7% ± 10.8%	73.5% ± 11.3%
Range	24%-78%	16%-56%	52%-90%

	Hepatoblastoma	Hepatocellular Carcinoma or Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies			
Ginkgo biloba extract (March 2005)	3/50	24/50	39/50
Isoeugenol (April 2002)	3/50	11/50	30/50
Kava kava extract (August 2004)	0/50	20/50	38/50
β-Myrcene (March 2002)	4/50	16/50	34/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	1/50	22/50	38/50
Pulegone (April 2003)	1/50	13/50	29/50
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	2/50	19/50	34/50
Total (%)	14/350 (4.0%)	125/350 (35.7%)	242/350 (69.1%)
Mean ± standard deviation	4.0% ± 2.8%	35.7% ± 9.5%	69.1% ± 8.0%
Range	2%-8%	22%-48%	58%-78%
Overall Historical Incidence: All Routes			
Total (%)	61/1,149 (5.3%)	433/1,149 (37.7%)	852/1,149 (74.2%)
Mean ± standard deviation	5.3% ± 7.1%	37.7% ± 11.1%	74.2% ± 11.5%
Range	0%-34%	18%-58%	52%-92%

[a] Data as of May 2011

TABLE C3b
Historical Incidence of Thyroid Gland Follicular Cell Adenoma in Control Male B6C3F1/N mice[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
Ginkgo biloba extract (March 2005)	0/49
Isoeugenol (April 2002)	0/50
Kava kava extract (August 2004)	0/50
β-Myrcene (March 2002)	0/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	1/50
Pulegone (April 2003)	0/50
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	0/50
Total (%)	1/349 (0.3%)
Mean ± standard deviation	0.3% ± 0.8%
Range	0%-2%
Overall Historical Incidence: All Routes	
Total (%)	7/1,143 (0.6%)
Mean ± standard deviation	0.6% ± 1.0%
Range	0%-2%

[a] Data as of May 2011

TABLE C3c
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
Ginkgo biloba extract (March 2005)	8/50	11/50	17/50
Isoeugenol (April 2002)	6/50	2/50	7/50
Kava kava extract (August 2004)	9/50	2/50	11/50
β-Myrcene (March 2002)	8/50	5/50	13/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	11/50	2/50	13/50
Pulegone (April 2003)	6/50	3/50	9/50
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	5/50	3/50	7/50
Total (%)	53/530 (15.1%)	28/350 (8.0%)	77/350 (22.0%)
Mean ± standard deviation	15.1% ± 4.1%	8.0% ± 6.5%	22.0% ± 7.3%
Range	10%-22%	4%-22%	14%-34%
Overall Historical Incidence: All Routes			
Total (%)	172/1,150 (15.0%)	144/1,150 (12.5%)	301/1,150 (26.2%)
Mean ± standard deviation	15.0% ± 6.9%	12.5% ± 7.1%	26.2% ± 6.3%
Range	2%-30%	4%-24%	14%-40%

[a] Data as of May 2011

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death			1	
Moribund	8	16	23	13
Natural deaths	8	7	5	14
Survivors				
Terminal kill	34	27	21	23
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(46)	(48)	(44)	(46)
Hyperplasia, cystic				1 (2%)
Inflammation	2 (4%)			
Intestine large, cecum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	3 (6%)		2 (4%)	1 (2%)
Epithelium, hyperplasia	1 (2%)			
Intestine large, colon	(50)	(50)	(50)	(50)
Epithelium, hyperplasia		1 (2%)		
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Ectopic tissue	1 (2%)			1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid				1 (2%)
Inflammation	3 (6%)		1 (2%)	
Epithelium, hyperplasia	3 (6%)		1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	7 (14%)	1 (2%)	6 (12%)	2 (4%)
Inflammation	3 (6%)		1 (2%)	
Mineralization	1 (2%)			
Epithelium, hyperplasia	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)			
Basophilic focus	15 (30%)	14 (28%)	5 (10%)	4 (8%)
Clear cell focus	21 (42%)	22 (44%)	14 (28%)	12 (24%)
Eosinophilic focus	34 (68%)	41 (82%)	36 (72%)	39 (78%)
Erythrophagocytosis		4 (8%)	11 (22%)	7 (14%)
Fatty change, focal	4 (8%)	3 (6%)		
Hematopoietic cell proliferation	4 (8%)	9 (18%)	12 (24%)	14 (28%)
Hepatodiaphragmatic nodule	1 (2%)			
Hypertrophy	3 (6%)	19 (38%)	35 (70%)	23 (46%)
Infiltration cellular, lymphoid	1 (2%)			
Inflammation	28 (56%)	35 (70%)	42 (84%)	39 (78%)
Mixed cell focus	15 (30%)	13 (26%)	12 (24%)	9 (18%)
Necrosis	9 (18%)	15 (30%)	17 (34%)	19 (38%)
Tension lipidosis	6 (12%)	1 (2%)		1 (2%)
Vacuolization cytoplasmic	16 (32%)	13 (26%)	14 (28%)	14 (28%)
Bile duct, hyperplasia		2 (4%)	1 (2%)	2 (4%)
Hepatocyte, hyperplasia			1 (2%)	
Vein, thrombosis			1 (2%)	
Mesentery	(5)	(6)	(3)	(4)
Inflammation		2 (33%)	2 (67%)	2 (50%)
Fat, necrosis	5 (100%)	4 (67%)		

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

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Atrophy	1 (2%)		1 (2%)	
Cyst			1 (2%)	
Infiltration cellular, lymphoid				2 (4%)
Inflammation		1 (2%)		2 (4%)
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid	5 (10%)	4 (8%)	5 (10%)	1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Infiltration cellular, mast cell			1 (2%)	
Inflammation	11 (22%)	24 (48%)	21 (42%)	45 (90%)
Mineralization		2 (4%)	1 (2%)	
Epithelium, erosion		2 (4%)	1 (2%)	3 (6%)
Epithelium, hyperkeratosis	11 (22%)	24 (48%)	24 (48%)	46 (92%)
Epithelium, hyperplasia	14 (28%)	27 (54%)	27 (54%)	45 (90%)
Epithelium, ulcer	7 (14%)	10 (20%)	12 (24%)	24 (48%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst	1 (2%)			1 (2%)
Inflammation	2 (4%)		1 (2%)	1 (2%)
Mineralization		2 (4%)	1 (2%)	
Epithelium, hyperplasia	4 (8%)	5 (10%)	7 (14%)	4 (8%)
Tooth	(50)	(50)	(50)	(50)
Dysplasia	46 (92%)	46 (92%)	40 (80%)	33 (66%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	38 (76%)	22 (44%)	27 (54%)	25 (50%)
Inflammation	1 (2%)	3 (6%)		
Mineralization	1 (2%)		2 (4%)	
Thrombosis	1 (2%)	1 (2%)		1 (2%)
Artery, inflammation		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia	1 (2%)			
Hypertrophy	9 (18%)		3 (6%)	1 (2%)
Subcapsular, hyperplasia	41 (82%)	39 (78%)	42 (84%)	43 (86%)
Adrenal medulla	(50)	(50)	(50)	(49)
Hyperplasia	2 (4%)	1 (2%)		2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	39 (78%)	38 (76%)	44 (88%)	35 (70%)
Parathyroid gland	(36)	(46)	(46)	(44)
Pituitary gland	(49)	(50)	(50)	(49)
Cyst				1 (2%)
Inflammation	1 (2%)			
Pars distalis, hyperplasia	2 (4%)		1 (2%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Endocrine System (continued)				
Thyroid gland	(49)	(49)	(50)	(50)
Cyst			1 (2%)	
Ultimobranchial, cyst	2 (4%)	2 (4%)	1 (2%)	
Ultimobranchial cyst, multiple	3 (6%)	1 (2%)		
Bilateral, ultimobranchial cyst	1 (2%)			
C-cell, hyperplasia	2 (4%)	5 (10%)		
Follicle, cyst	2 (4%)	1 (2%)		
Follicle, hyperplasia	2 (4%)	1 (2%)	7 (14%)	25 (50%)
Follicle, ultimobranchial cyst	2 (4%)			
Follicular cell, degeneration		1 (2%)		
Follicular cell, hypertrophy	2 (4%)		2 (4%)	38 (76%)
General Body System				
Peritoneum	(0)	(1)	(2)	(2)
Inflammation		1 (100%)	2 (100%)	2 (100%)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Infiltration cellular, lymphoid	3 (6%)	4 (8%)	3 (6%)	2 (4%)
Inflammation	1 (2%)	3 (6%)	2 (4%)	3 (6%)
Preputial gland	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Ectasia	4 (8%)	3 (6%)	5 (10%)	8 (16%)
Fibrosis				1 (2%)
Infiltration cellular, lymphoid	1 (2%)			
Inflammation	30 (60%)	42 (84%)	49 (98%)	46 (92%)
Prostate	(50)	(50)	(49)	(50)
Hyperplasia	7 (14%)	3 (6%)	1 (2%)	1 (2%)
Infiltration cellular, lymphoid		3 (6%)	1 (2%)	
Inflammation	3 (6%)	5 (10%)	4 (8%)	4 (8%)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation	2 (4%)		1 (2%)	
Hyperplasia	1 (2%)		1 (2%)	
Inflammation	2 (4%)	2 (4%)		1 (2%)
Testes	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		2 (4%)
Mineralization		3 (6%)		2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia	48 (96%)	49 (98%)	49 (98%)	50 (100%)
Lymph node	(11)	(15)	(18)	(14)
Bronchial, hyperplasia, lymphoid	6 (55%)	2 (13%)	1 (6%)	4 (29%)
Bronchial, infiltration cellular, histiocyte				1 (7%)
Inguinal, hyperplasia, lymphoid	6 (55%)	10 (67%)	16 (89%)	11 (79%)
Mediastinal, hyperplasia, lymphoid		1 (7%)		
Mediastinal, infiltration cellular, histiocyte			1 (6%)	
Lymph node, mandibular	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	17 (34%)	14 (28%)	16 (32%)	5 (10%)
Infiltration cellular, histiocyte			1 (2%)	1 (2%)
Lymph node, mesenteric	(50)	(49)	(49)	(50)
Hyperplasia, lymphoid	6 (12%)	5 (10%)	10 (20%)	1 (2%)
Inflammation		1 (2%)	1 (2%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Hematopoietic System (continued)				
Spleen	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Hematopoietic cell proliferation	49 (98%)	47 (94%)	50 (100%)	50 (100%)
Hyperplasia				1 (2%)
Hyperplasia, lymphoid	9 (18%)	12 (24%)	6 (12%)	8 (16%)
Lymphoid follicle, atrophy		1 (2%)		
Thymus	(50)	(47)	(50)	(45)
Atrophy	1 (2%)			
Hyperplasia, lymphoid	10 (20%)	10 (21%)	19 (38%)	10 (22%)
Necrosis		1 (2%)		
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Dermis, fibrosis	2 (4%)		1 (2%)	
Dermis, inflammation	2 (4%)	2 (4%)	1 (2%)	
Dermis, subcutaneous tissue, inflammation		1 (2%)		
Epidermis, hyperkeratosis	1 (2%)	1 (2%)		
Epidermis, hyperplasia	2 (4%)	1 (2%)	1 (2%)	
Epidermis, ulcer	2 (4%)	2 (4%)	1 (2%)	
Subcutaneous tissue, inflammation		2 (4%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperplasia			1 (2%)	
Vertebra, fracture			1 (2%)	
Skeletal muscle	(1)	(1)	(1)	(1)
Cyst				1 (100%)
Hemorrhage			1 (100%)	
Inflammation	1 (100%)			1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Infiltration cellular, lymphoid	2 (4%)			1 (2%)
Peripheral nerve	(0)	(0)	(1)	(0)
Spinal, hemorrhage			1 (100%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Infiltration cellular, histiocyte	1 (2%)			
Inflammation	5 (10%)	1 (2%)	6 (12%)	3 (6%)
Metaplasia, osseous				1 (2%)
Mineralization	2 (4%)	1 (2%)		1 (2%)
Pigmentation			1 (2%)	
Alveolar epithelium, hyperplasia	4 (8%)	3 (6%)	1 (2%)	1 (2%)
Alveolus, infiltration cellular, histiocyte	10 (20%)	7 (14%)	11 (22%)	5 (10%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Respiratory System (continued)				
Nose	(50)	(50)	(50)	(50)
Inflammation,	7 (14%)	16 (32%)	5 (10%)	9 (18%)
Glands, metaplasia	1 (2%)			
Glands, metaplasia, respiratory	3 (6%)	3 (6%)	2 (4%)	1 (2%)
Olfactory epithelium, accumulation, hyaline droplet	18 (36%)	16 (32%)	15 (30%)	28 (56%)
Olfactory epithelium, atrophy		3 (6%)		
Olfactory epithelium, degeneration	1 (2%)			
Olfactory epithelium, hyperplasia	2 (4%)			1 (2%)
Olfactory epithelium, metaplasia	1 (2%)			
Olfactory epithelium, metaplasia, respiratory	3 (6%)	3 (6%)		
Olfactory epithelium, pigmentation		1 (2%)	3 (6%)	13 (26%)
Respiratory epithelium, hyperplasia	40 (80%)	38 (76%)	37 (74%)	33 (66%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(1)	(0)	(0)	(1)
Eye	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Cataract			1 (2%)	
Inflammation	1 (2%)			
Cornea, hyperplasia, squamous	1 (2%)	1 (2%)	3 (6%)	
Cornea, inflammation	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Cyst				1 (2%)
Fibrosis		1 (2%)		
Hyperplasia	3 (6%)	5 (10%)	3 (6%)	2 (4%)
Infiltration cellular, lymphoid		1 (2%)	1 (2%)	
Mineralization		1 (2%)		
Pigmentation	1 (2%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet		1 (2%)		
Cyst	6 (12%)	6 (12%)	8 (16%)	5 (10%)
Infarct	2 (4%)	2 (4%)		
Infiltration cellular, lymphoid	2 (4%)	1 (2%)	1 (2%)	
Inflammation	2 (4%)	4 (8%)	1 (2%)	1 (2%)
Metaplasia, osseous	3 (6%)	4 (8%)	4 (8%)	
Mineralization	40 (80%)	40 (80%)	38 (76%)	21 (42%)
Nephropathy	49 (98%)	45 (90%)	44 (88%)	39 (78%)
Pigmentation	3 (6%)	15 (30%)	26 (52%)	19 (38%)
Pelvis, dilatation			1 (2%)	
Renal tubule, hyperplasia	1 (2%)	1 (2%)		
Renal tubule, necrosis		1 (2%)		
Urethra	(0)	(1)	(0)	(0)
Bulbourethral gland, inflammation		1 (100%)		
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Inflammation	2 (4%)	2 (4%)		1 (2%)

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF *GINKGO BILOBA* EXTRACT

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	5	2	1	
Moribund	8	8	3	3
Natural death	6	4	3	11
Survivors				
Terminal kill	31	36	43	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(50)	(48)	(48)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Fibrous histiocytoma				1 (2%)
Hemangiosarcoma		1 (2%)	1 (2%)	
Hepatoblastoma	1 (2%)	1 (2%)	7 (14%)	7 (14%)
Hepatoblastoma, multiple			1 (2%)	4 (8%)
Hepatocellular adenoma	7 (14%)	7 (14%)	4 (8%)	5 (10%)
Hepatocellular adenoma, multiple	10 (20%)	30 (60%)	37 (74%)	43 (86%)
Hepatocellular carcinoma	7 (14%)	8 (16%)	10 (20%)	13 (26%)
Hepatocellular carcinoma, multiple	2 (4%)	2 (4%)	5 (10%)	31 (62%)
Osteosarcoma, metastatic, bone				1 (2%)
Mesentery	(3)	(5)	(3)	(4)
Oral mucosa	(1)	(0)	(0)	(0)
Gingival, squamous cell carcinoma	1 (100%)			
Pancreas	(50)	(49)	(50)	(50)
Fibrous histiocytoma				1 (2%)
Salivary glands	(50)	(50)	(49)	(50)
Sarcoma, metastatic, skin		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	2 (4%)	1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Tooth	(50)	(50)	(50)	(50)
Cardiovascular System				
Blood vessel	(50)	(49)	(50)	(50)
Heart	(50)	(50)	(50)	(50)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Fibrous histiocytoma				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign		1 (2%)		
Pheochromocytoma malignant		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(47)	(46)	(45)	(45)
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, adenoma	4 (8%)	2 (4%)	1 (2%)	
Pars intermedia, adenoma	1 (2%)			1 (2%)
Thyroid gland	(49)	(48)	(49)	(48)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(49)
Ovary	(50)	(50)	(49)	(50)
Choriocarcinoma	1 (2%)			
Cystadenoma	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Fibrous histiocytoma				1 (2%)
Hemangioma				1 (2%)
Hemangiosarcoma	1 (2%)			
Teratoma benign			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Polyp stromal	1 (2%)			
Sarcoma stromal	1 (2%)			
Endometrium, polyp stromal			1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(16)	(11)	(11)	(6)
Lymph node, bronchial	(0)	(0)	(0)	(1)
Lymph node, mandibular	(49)	(50)	(49)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			
Thymus	(50)	(49)	(49)	(50)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)	1 (2%)	
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	1 (2%)			
Keratoacanthoma				1 (2%)
Melanoma malignant	1 (2%)			
Sarcoma	1 (2%)	1 (2%)		
Subcutaneous tissue, sarcoma	2 (4%)	2 (4%)		
Tail, fibrous histiocytoma			1 (2%)	
Tail, melanoma malignant		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma				1 (2%)
Skeletal muscle	(1)	(1)	(0)	(0)
Hemangiosarcoma	1 (100%)			
Sarcoma		1 (100%)		
Nervous System				
Brain	(50)	(50)	(49)	(50)
Peripheral nerve	(1)	(1)	(1)	(0)
Spinal cord	(0)	(1)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma				1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Hepatoblastoma, metastatic, liver			1 (2%)	
Hepatocellular carcinoma, metastatic, liver	3 (6%)	3 (6%)	2 (4%)	5 (10%)
Osteosarcoma, metastatic, bone				1 (2%)
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		
Sarcoma, metastatic, skin	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Eye	(50)	(50)	(49)	(50)
Harderian gland	(50)	(50)	(49)	(50)
Adenoma	5 (10%)	3 (6%)	2 (4%)	10 (20%)
Carcinoma	1 (2%)		4 (8%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Fibrous histiocytoma				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Fibrous histiocytoma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Leukemia myeloid				1 (2%)
Lymphoma malignant	11 (22%)	9 (18%)	7 (14%)	2 (4%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	35	46	46	49
Total primary neoplasms	67	79	90	135
Total animals with benign neoplasms	25	39	41	48
Total benign neoplasms	32	48	48	63
Total animals with malignant neoplasms	28	26	30	47
Total malignant neoplasms	35	31	42	72
Total animals with metastatic neoplasms	4	5	3	6
Total metastatic neoplasms	4	5	3	7

[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
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/50 (10%)	3/50 (6%)	2/50 (4%)	10/50 (20%)
Adjusted rate[b]	12.2%	6.6%	4.3%	21.6%
Terminal rate[c]	4/31 (13%)	2/36 (6%)	2/43 (5%)	8/36 (22%)
First incidence (days)	653	704	727 (T)	609
Poly-3 test[d]	P=0.018	P=0.299N	P=0.166N	P=0.192
Harderian Gland: Carcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	4/50 (8%)	1/50 (2%)
Adjusted rate	2.5%	0.0%	8.5%	2.2%
Terminal rate	1/31 (3%)	0/36 (0%)	3/43 (7%)	1/36 (3%)
First incidence (days)	727 (T)	—[e]	587	727 (T)
Poly-3 test	P=0.641	P=0.478N	P=0.228	P=0.734N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	3/50 (6%)	6/50 (12%)	10/50 (20%)
Adjusted rate	14.7%	6.6%	12.7%	21.6%
Terminal rate	5/31 (16%)	2/36 (6%)	5/43 (12%)	8/36 (22%)
First incidence (days)	653	704	587	609
Poly-3 test	P=0.061	P=0.191N	P=0.517N	P=0.290
Liver: Hepatocellular Adenoma				
Overall rate	17/50 (34%)	37/50 (74%)	41/50 (82%)	48/50 (96%)
Adjusted rate	41.0%	78.3%	86.9%	96.0%
Terminal rate	14/31 (45%)	29/36 (81%)	38/43 (88%)	34/36 (94%)
First incidence (days)	589	596	628	497
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rate	9/50 (18%)	10/50 (20%)	15/50 (30%)	44/50 (88%)
Adjusted rate	21.7%	21.8%	32.1%	91.7%
Terminal rate	5/31 (16%)	8/36 (22%)	14/43 (33%)	34/36 (94%)
First incidence (days)	653	656	718	593
Poly-3 test	P<0.001	P=0.596	P=0.197	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	20/50 (40%)	39/50 (78%)	41/50 (82%)	49/50 (98%)
Adjusted rate	47.7%	82.1%	86.9%	98.0%
Terminal rate	15/31 (48%)	30/36 (83%)	38/43 (88%)	35/36 (97%)
First incidence (days)	589	596	628	497
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	1/50 (2%)	8/50 (16%)	11/50 (22%)
Adjusted rate	2.5%	2.2%	17.1%	23.8%
Terminal rate	1/31 (3%)	1/36 (3%)	8/43 (19%)	8/36 (22%)
First incidence (days)	727 (T)	727 (T)	727 (T)	609
Poly-3 test	P<0.001	P=0.735N	P=0.028	P=0.004
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	10/50 (20%)	11/50 (22%)	18/50 (36%)	44/50 (88%)
Adjusted rate	24.1%	24.0%	38.5%	91.7%
Terminal rate	6/31 (19%)	9/36 (25%)	17/43 (40%)	34/36 (94%)
First incidence (days)	653	656	718	593
Poly-3 test	P<0.001	P=0.594N	P=0.110	P<0.001

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	1/50 (2%)	2/50 (4%)	2/50 (4%)	4/50 (8%)
Adjusted rate	2.5%	4.4%	4.3%	8.7%
Terminal rate	0/31 (0%)	1/36 (3%)	2/43 (5%)	3/36 (8%)
First incidence (days)	659	685	727 (T)	609
Poly-3 test	P=0.149	P=0.538	P=0.547	P=0.217
Ovary: Cystadenoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/49 (2%)	1/50 (2%)
Adjusted rate	4.9%	6.5%	2.2%	2.2%
Terminal rate	1/31 (3%)	1/36 (3%)	1/43 (2%)	1/36 (3%)
First incidence (days)	531	659	727 (T)	727(T)
Poly-3 test	P=0.294N	P=0.548	P=0.464N	P=0.465N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/50 (8%)	2/50 (4%)	1/49 (2%)	0/50 (0%)
Adjusted rate	9.9%	4.4%	2.2%	0.0%
Terminal rate	4/31 (13%)	2/36 (6%)	1/43 (2%)	0/36 (0%)
First incidence (days)	727 (T)	727 (T)	727 (T)	—
Poly-3 test	P=0.056N	P=0.287N	P=0.145N	P=0.047N
Skin: Sarcoma				
Overall rate	3/50 (6%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	7.3%	6.5%	0.0%	0.0%
Terminal rate	1/31 (3%)	1/36 (3%)	0/43 (0%)	0/36 (0%)
First incidence (days)	659	447	—	—
Poly-3 test	P=0.058N	P=0.604N	P=0.097N	P=0.101N
Skin: Fibrous Histiocytoma or Sarcoma				
Overall rate	3/50 (6%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	7.3%	6.5%	2.1%	0.0%
Terminal rate	1/31 (3%)	1/36 (3%)	1/43 (2%)	0/36 (0%)
First incidence (days)	659	447	727 (T)	—
Poly-3 test	P=0.065N	P=0.604N	P=0.260N	P=0.101N
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	2.5%	2.2%	6.3%	6.5%
Terminal rate	0/31 (0%)	1/36 (3%)	2/43 (5%)	1/36 (3%)
First incidence (days)	700	727 (T)	414	589
Poly-3 test	P=0.250	P=0.736N	P=0.363	P=0.353
All Organs: Benign Neoplasms				
Overall rate	25/50 (50%)	39/50 (78%)	41/50 (82%)	48/50 (96%)
Adjusted rate	58.8%	82.1%	86.9%	96.0%
Terminal rate	18/31 (58%)	30/36 (83%)	38/43 (88%)	34/36 (94%)
First incidence (days)	531	596	628	497
Poly-3 test	P<0.001	P=0.010	P=0.002	P<0.001
All Organs: Malignant Neoplasms				
Overall rate	28/50 (56%)	26/50 (52%)	30/50 (60%)	47/50 (94%)
Adjusted rate	64.9%	53.8%	62.4%	95.3%
Terminal rate	18/31 (58%)	17/36 (47%)	26/43 (61%)	34/36 (94%)
First incidence (days)	476	447	414	497
Poly-3 test	P<0.001	P=0.191N	P=0.487N	P<0.001

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	35/50 (70%)	46/50 (92%)	46/50 (92%)	49/50 (98%)
Adjusted rate	79.2%	93.3%	94.9%	98.0%
Terminal rate	23/31 (74%)	33/36 (92%)	41/43 (95%)	35/36 (97%)
First incidence (days)	476	447	414	497
Poly-3 test	P=0.012	P=0.041	P=0.021	P=0.003

(T) Terminal kill

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

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value 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 Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

TABLE D3
Historical Incidence of Liver Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
Ginkgo biloba extract (March 2005)	17/50	9/50	20/50
Isoeugenol (April 2002)	11/49	3/49	13/49
Kava kava extract (August 2004)	8/50	3/50	10/50
β-Myrcene (March 2002)	6/50	1/50	7/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	17/50	6/50	20/50
Pulegone (April 2003)	13/49	5/49	17/49
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	3/49	2/49	4/49
Total (%)	75/347 (21.6%)	29/347 (8.4%)	91/347 (26.2%)
Mean ± standard deviation	21.6% ± 10.8%	8.3% ± 5.5%	26.2% ± 12.7%
Range	6%-34%	2%-18%	8%-40%
Overall Historical Incidence: All Routes			
Total (%)	380/1,195 (31.8%)	144/1,195 (12.1%)	444/1,195 (37.2%)
Mean ± standard deviation	31.8% ± 21.4%	12.1% ± 10.8%	37.2% ± 22.9%
Range	2%-78%	0%-46%	6%-82%

	Hepatoblastoma	Hepatocellular Carcinoma or Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies			
Ginkgo biloba extract (March 2005)	1/50	10/50	20/50
Isoeugenol (April 2002)	0/49	3/49	13/49
Kava kava extract (August 2004)	0/50	3/50	10/50
β-Myrcene (March 2002)	0/50	1/50	7/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	0/50	6/50	20/50
Pulegone (April 2003)	0/49	5/49	17/49
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	0/49	2/49	4/49
Total (%)	1/347 (0.3%)	30/347 (8.7%)	91/347 (26.2%)
Mean ± standard deviation	0.3% ± 0.8%	8.6% ± 6.1%	26.2% ± 12.7%
Range	0%-2%	2%-20%	8%-40%
Overall Historical Incidence: All Routes			
Total (%)	4/1,195 (0.3%)	148/1,195 (12.4%)	444/1,195 (37.2%)
Mean ± standard deviation	0.3% ± 0.8%	12.4% ± 11.2%	37.2% ± 22.9%
Range	0%-2%	0%-46%	6%-82%

[a] Data as of May 2011

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	5	2	1	
Moribund	8	8	3	3
Natural death	6	4	3	11
Survivors				
Terminal kill	31	36	43	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	5 (10%)		1 (2%)	
Perforation	4 (8%)			
Gallbladder	(50)	(50)	(48)	(48)
Cyst		3 (6%)		
Intestine large, cecum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid				1 (2%)
Inflammation			1 (2%)	
Necrosis		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Epithelium, hyperplasia	1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Epithelium, hyperplasia	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Basophilic focus	7 (14%)	8 (16%)	9 (18%)	9 (18%)
Clear cell focus	1 (2%)	3 (6%)	2 (4%)	6 (12%)
Eosinophilic focus	26 (52%)	39 (78%)	43 (86%)	45 (90%)
Erythrophagocytosis		3 (6%)	7 (14%)	16 (32%)
Fatty change, focal	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Hematopoietic cell proliferation	14 (28%)	12 (24%)	9 (18%)	4 (8%)
Hypertrophy		18 (36%)	37 (74%)	37 (74%)
Inflammation	38 (76%)	45 (90%)	46 (92%)	41 (82%)
Mineralization				1 (2%)
Mixed cell focus	7 (14%)	27 (54%)	31 (62%)	31 (62%)
Necrosis	4 (8%)	2 (4%)	6 (12%)	11 (22%)
Tension lipidosis	5 (10%)	11 (22%)	10 (20%)	3 (6%)
Vacuolization, cytoplasmic	18 (36%)	38 (76%)	44 (88%)	35 (70%)
Bile duct, cyst		2 (4%)	1 (2%)	
Mesentery	(3)	(5)	(3)	(4)
Inflammation			1 (33%)	1 (25%)
Fat, necrosis	2 (67%)	5 (100%)	3 (100%)	2 (50%)
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(50)	(49)	(50)	(50)
Atrophy	1 (2%)	4 (8%)	4 (8%)	1 (2%)
Cyst	1 (2%)			
Infiltration cellular, lymphoid		3 (6%)	1 (2%)	
Inflammation			1 (2%)	2 (4%)
Acinus, hyperplasia			1 (2%)	
Acinus, hypertrophy	1 (2%)			
Duct, cyst		1 (2%)	3 (6%)	1 (2%)

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

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Alimentary System (continued)				
Salivary glands	(50)	(50)	(49)	(50)
Infiltration cellular, lymphoid	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation	4 (8%)	6 (12%)	5 (10%)	19 (38%)
Mineralization		1 (2%)		
Epithelium, erosion		1 (2%)		
Epithelium, hperkeratosis	3 (6%)	11 (22%)	5 (10%)	20 (40%)
Epithelium, hyperplasia	8 (16%)	18 (36%)	11 (22%)	20 (40%)
Epithelium, ulcer	1 (2%)	1 (2%)	1 (2%)	11 (22%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst		1 (2%)	2 (4%)	1 (2%)
Inflammation		2 (4%)	1 (2%)	3 (6%)
Mineralization	1 (2%)			
Epithelium, hyperplasia	2 (4%)	2 (4%)	1 (2%)	5 (10%)
Tooth	(50)	(50)	(50)	(50)
Dysplasia		4 (8%)	3 (6%)	
Cardiovascular System				
Blood vessel	(50)	(49)	(50)	(50)
Mineralization	1 (2%)	1 (2%)		
Aorta, inflammation	1 (2%)			
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	7 (14%)	5 (10%)	5 (10%)	7 (14%)
Inflammation	2 (4%)	1 (2%)		
Mineralization	2 (4%)	2 (4%)	3 (6%)	
Necrosis		1 (2%)		
Epicardium, inflammation	2 (4%)		1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Hematopoietic cell proliferation	5 (10%)	1 (2%)	3 (6%)	3 (6%)
Hypertrophy	1 (2%)			
Infiltration cellular, lymphoid			1 (2%)	1 (2%)
Subcapsular, hyperplasia	46 (92%)	50 (100%)	49 (98%)	50 (100%)
Adrenal Medulla	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Hypertrophy				1 (2%)
Islets, Pancreatic	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia	7 (14%)	11 (22%)	11 (22%)	9 (18%)
Parathyroid gland	(47)	(46)	(45)	(45)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Infiltration cellular, lymphoid			2 (4%)	
Pituitary gland	(50)	(50)	(49)	(50)
Angiectasis		1 (2%)		
Pars distalis, hyperplasia	14 (28%)	20 (40%)	22 (45%)	10 (20%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Endocrine System (continued)				
Thyroid gland	(49)	(48)	(49)	(48)
Ultimobranchial cyst	3 (6%)		3 (6%)	3 (6%)
Ultimobranchial cyst, multiple	11 (22%)	5 (10%)	6 (12%)	
Bilateral, ultimobronchial cyst		1 (2%)		
Bilateral, ultimobronchial cyst, multiple		1 (2%)		
C-cell, hyperplasia	3 (6%)	6 (13%)	5 (10%)	1 (2%)
Follicle, cyst	1 (2%)		2 (4%)	1 (2%)
Follicle cyst, multiple	1 (2%)	1 (2%)		
Follicle, hyperplasia			4 (8%)	
Follicle cell, cyst, multiple	1 (2%)			
Follicular cell, degeneration			1 (2%)	
Follicular cell, hypertrophy	1 (2%)	5 (10%)	9 (18%)	39 (81%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(49)
Inflammation	4 (8%)	2 (4%)	1 (2%)	
Ovary	(50)	(50)	(49)	(50)
Angiectasis	2 (4%)			2 (4%)
Cyst	8 (16%)	10 (20%)	17 (35%)	11 (22%)
Hemorrhage, chronic		1 (2%)		
Mineralization	1 (2%)		1 (2%)	
Thrombosis	1 (2%)		1 (2%)	
Germinal epithelium, hyperplasia			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	1 (2%)
Dysplasia				1 (2%)
Fibrosis	1 (2%)			
Hemorrhage			1 (2%)	
Inflammation	7 (14%)	10 (20%)	3 (6%)	2 (4%)
Malformation		1 (2%)		
Artery, dysplasia			1 (2%)	
Endometrium, decidual reaction	1 (2%)			
Endometrium, hyperplasia	1 (2%)			
Endometrium, hyperplasia, cystic	35 (70%)	37 (74%)	35 (70%)	30 (60%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	38 (76%)	43 (86%)	42 (84%)	36 (72%)
Necrosis		1 (2%)		
Lymph node	(16)	(11)	(11)	(6)
Bronchial, hyperplasia, lymphoid	3 (19%)	3 (27%)	2 (18%)	1 (17%)
Inguinal, hyperplasia, lymphoid	7 (44%)	2 (18%)	2 (18%)	3 (50%)
Mediastinal, hyperplasia, lymphoid		2 (18%)		1 (17%)
Pancreatic, angiectasis	1 (6%)			
Pancreatic, hyperplasia, lymphoid	1 (6%)		1 (9%)	
Renal, angiectasis	1 (6%)			
Renal, ectasia			1 (9%)	
Renal, hemorrhage, chronic			1 (9%)	
Renal, sinus, ectasia			1 (9%)	
Lymph node, bronchial	(0)	(0)	(0)	(1)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(49)	(50)	(49)	(50)
Angiectasis			1 (2%)	
Hyperplasia, lymphoid	6 (12%)	15 (30%)	10 (20%)	7 (14%)
Inflammation	1 (2%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	6 (12%)	5 (10%)	2 (4%)	
Inflammation			1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	48 (96%)	48 (96%)	50 (100%)	50 (100%)
Hyperplasia, lymphoid	20 (40%)	33 (66%)	25 (50%)	29 (58%)
Mineralization		1 (2%)		
Necrosis			1 (2%)	
Pigmentation	4 (8%)			
Lymphoid follicle, atrophy			1 (2%)	1 (2%)
Thymus	(50)	(49)	(49)	(50)
Angiectasis				1 (2%)
Hyperplasia, lymphoid	18 (36%)	16 (33%)	23 (47%)	13 (26%)
Inflammation			1 (2%)	
Necrosis	3 (6%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)			
Inflammation			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Dermis, inflammation	1 (2%)		1 (2%)	
Epidermis, hyperplasia	1 (2%)			
Epidermis, ulcer	1 (2%)			
Sebaceous gland, hyperplasia	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fracture		2 (4%)		
Osteopetrosis			1 (2%)	
Osteoporosis	7 (14%)	16 (32%)	17 (34%)	6 (12%)
Synovial tissue, inflammation		1 (2%)		
Skeletal muscle	(1)	(1)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(49)	(50)
Gliosis	1 (2%)	1 (2%)		
Hemorrhage		1 (2%)		
Peripheral nerve	(1)	(1)	(1)	(0)
Sciatic, demyelination	1 (100%)			
Spinal, demyelination	1 (100%)			
Spinal cord	(0)	(1)	(1)	(0)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Hemorrhage		2 (4%)		
Infiltration cellular, histiocyte		3 (6%)	2 (4%)	3 (6%)
Inflammation		5 (10%)	3 (6%)	7 (14%)
Metaplasia, osseous	1 (2%)			1 (2%)
Mineralization		1 (2%)		
Thrombosis	1 (2%)			
Vacuolization, cytoplasmic				1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)		
Alveolar epithelium, inflammation	1 (2%)			
Arteriole, hyperplasia				3 (6%)
Bronchiole, hyperplasia		1 (2%)		
Pleura, inflammation	5 (10%)		1 (2%)	
Smooth muscle, proliferation				1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation,	4 (8%)	5 (10%)	4 (8%)	6 (12%)
Glands, metaplasia	3 (6%)	4 (8%)	5 (10%)	2 (4%)
Olfactory epithelium, accumulation, hyaline droplet	5 (10%)	3 (6%)	12 (24%)	17 (34%)
Olfactory epithelium, atrophy			1 (2%)	1 (2%)
Olfactory epithelium, degeneration	3 (6%)			
Olfactory epithelium, hyperplasia	1 (2%)			
Olfactory epithelium, metaplasia	1 (2%)			
Olfactory epithelium, pigmentation		1 (2%)	6 (12%)	13 (26%)
Respiratory epithelium, hyperplasia	15 (30%)	15 (30%)	18 (36%)	15 (30%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Eye	(50)	(50)	(49)	(50)
Cataract		1 (2%)	1 (2%)	
Phthisis bulbi		1 (2%)		
Anterior chamber, inflammation	1 (2%)			1 (2%)
Cornea, inflammation	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Harderian gland	(50)	(50)	(49)	(50)
Fibrosis			1 (2%)	
Hyperplasia	2 (4%)	1 (2%)	2 (4%)	4 (8%)
Infiltration cellular, lymphoid	1 (2%)			1 (2%)
Inflammation			1 (2%)	1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	200 mg/kg	600 mg/kg	2,000 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet			1 (2%)	
Cyst	1 (2%)		3 (6%)	3 (6%)
Dilatation		1 (2%)		
Glomerulopathy				1 (2%)
Infarct	1 (2%)		1 (2%)	
Infiltration cellular, lymphoid				1 (2%)
Inflammation	1 (2%)		2 (4%)	
Metaplasia, osseous	1 (2%)	1 (2%)	1 (2%)	
Mineralization	11 (22%)	8 (16%)	3 (6%)	10 (20%)
Nephropathy	24 (48%)	17 (34%)	15 (30%)	14 (28%)
Pigmentation			2 (4%)	1 (2%)
Glomerulus, amyloid deposition	1 (2%)			
Pelvis, dilatation				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid	6 (12%)	2 (4%)	2 (4%)	8 (16%)
Inflammation			1 (2%)	

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Testing procedures were modified from those reported by Zeiger *et al.* (1992). *Ginkgo biloba* extract (lot 020703; the same chemical lot that was used in the 3-month and 2-year studies) was sent to the laboratory as a coded aliquot. It was incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 and *Escherichia coli* (WP2 *uvrA*/pKM101) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat 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 *Ginkgo biloba* extract. The high dose was limited by experimental design to 10,000 µg/plate. All trials were repeated.

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, is not reproducible, or is 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 is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs; mature erythrocytes) in each of nine or 10 animals per dose group. In addition, the percentage of polychromatic erythrocytes (PCEs; reticulocytes) in 1,000 total erythrocytes per animal in the peripheral blood was scored for each dose group as a measure of bone marrow toxicity.

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 NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the vehicle control group. 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 dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month 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 magnitudes of those effects.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to

pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

Ginkgo biloba extract (1,000 to 10,000 µg/plate) was mutagenic in *S. typhimurium* strains TA98 and TA100 and in *E. coli* strain WP2 *uvrA*/pKM101, with and without 10% induced rat liver S9 mix (Table E1). No increase in the frequency of micronucleated erythrocytes was observed in peripheral blood of male B6C3F1/N mice administered *Ginkgo biloba* extract (125 to 2,000 mg/kg per day) for 3 months by gavage (Table E2). In female mice administered these doses, the results of the micronucleus test were judged to be equivocal based on a significant trend test and no individual dose groups being significantly elevated over the vehicle control group (Table E2). No significant alterations in the percentages of PCEs were seen in female mice, suggesting that exposure to *Ginkgo biloba* extract did not cause bone marrow toxicity in the females; in contrast, a significant dose-related decrease in the percentage of circulating reticulocytes was observed in male mice, suggesting that in males, increasing doses of *Ginkgo biloba* extract induced bone marrow toxicity (Table E2).

TABLE E1
Mutagenicity of *Ginkgo biloba* Extract in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100	0	59 ± 2	67 ± 7	69 ± 2	78 ± 2
	1,000	81 ± 3	55 ± 5	104 ± 4	114 ± 7
	2,500	93 ± 8	55 ± 9	106 ± 5	125 ± 9
	5,000	101 ± 3	67 ± 4	126 ± 6	131 ± 10
	7,500	103 ± 4	116 ± 1	132 ± 9	142 ± 6
	10,000	159 ± 10	130 ± 5	139 ± 17	172 ± 4
Trial summary		Positive	Positive	Positive	Positive
Positive control[b]		421 ± 20	769 ± 63	827 ± 18	830 ± 18
TA98	0	20 ± 2	21 ± 0	28 ± 2	33 ± 4
	1,000	34 ± 5	32 ± 5	94 ± 8	94 ± 5
	2,500	49 ± 4	39 ± 3	131 ± 27	146 ± 14
	5,000	58 ± 4	53 ± 3	145 ± 9	193 ± 16
	7,500	72 ± 5	64 ± 5	221 ± 11	239 ± 3
	10,000	66 ± 9	46 ± 4	243 ± 9	285 ± 23
Trial summary		Positive	Positive	Positive	Positive
Positive control		629 ± 16	649 ± 13	1,112 ± 65	951 ± 41
***Escherichia coli* WP2 *uvrA*/pKM101**					
	0	125 ± 3	149 ± 7	199 ± 17	207 ± 7
	1,000	144 ± 8	203 ± 15	241 ± 15	222 ± 8
	2,500	169 ± 2	260 ± 19	262 ± 11	303 ± 20
	5,000	165 ± 8	249 ± 16	259 ± 23	253 ± 13
	7,500	171 ± 10	258 ± 16	302 ± 2	262 ± 19
	10,000	230 ± 11	268 ± 7	306 ± 8	327 ± 43
Trial summary		Positive	Positive	Positive	Positive
Positive control		2,161 ± 37	859 ± 52	1,250 ± 39	1,015 ± 12

[a] The detailed protocol is presented by Zeiger *et al*. (1992); SITEK Research Laboratories used a modification of this protocol and the same lot (020703) that was used in the 3-month and 2-year studies. 0 µg/plate was the solvent control. Data are presented as revertants/plate (mean ± standard error) from three plates.

[b] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of *Ginkgo biloba* Extract by Gavage for 3 Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Corn oil[d]	0	10	2.25 ± 0.25		3.970 ± 0.21
Ginkgo biloba extract	125	10	2.40 ± 0.31	0.3777	3.720 ± 0.24
	250	10	3.00 ± 0.36	0.0714	3.370 ± 0.22
	500	10	2.90 ± 0.36	0.0998	3.020 ± 0.14
	1,000	10	2.00 ± 0.20	0.7064	2.570 ± 0.09
	2,000	10	2.60 ± 0.34	0.2384	2.160 ± 0.17
			P=0.556[e]		
Female					
Corn oil	0	10	1.05 ± 0.19		3.170 ± 0.17
Ginkgo biloba extract	125	10	1.25 ± 0.20	0.2776	3.940 ± 0.30
	250	10	1.25 ± 0.37	0.2776	3.330 ± 0.30
	500	10	1.75 ± 0.37	0.0306	3.010 ± 0.24
	1,000	9	1.89 ± 0.31	0.0159	2.567 ± 0.19
	2,000	10	2.05 ± 0.36	0.0055	2.600 ± 0.18
			P=0.002		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; dosed group values are significant at P≤0.005
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	9	10	9
Day 23	10	9	10	10	10	9
Week 14	10	10	10	10	9	10
Hematocrit (%)						
Day 4	44.1 ± 0.6	43.6 ± 0.6	43.5 ± 0.5	43.7 ± 0.9	43.0 ± 0.8	42.9 ± 0.6
Day 23	45.3 ± 0.5	45.7 ± 0.5	47.5 ± 0.7*	45.2 ± 0.9	45.1 ± 0.6	46.0 ± 0.4
Week 14	49.2 ± 0.5	47.6 ± 0.5	47.9 ± 0.4	47.6 ± 0.5	47.4 ± 0.6	47.8 ± 0.5
Hemoglobin (g/dL)						
Day 4	13.3 ± 0.2	13.4 ± 0.2	13.2 ± 0.2	13.3 ± 0.2	13.1 ± 0.3	13.0 ± 0.2
Day 23	15.0 ± 0.1	15.4 ± 0.2	15.8 ± 0.2*	15.1 ± 0.2	15.0 ± 0.2	15.4 ± 0.2
Week 14	15.6 ± 0.1	15.2 ± 0.2	15.0 ± 0.2*	15.1 ± 0.2	15.0 ± 0.1*	15.1 ± 0.2
Erythrocytes (10^6/μL)						
Day 4	7.23 ± 0.11	7.24 ± 0.09	7.20 ± 0.10	7.14 ± 0.12	7.09 ± 0.16	7.08 ± 0.09
Day 23	7.65 ± 0.08	7.70 ± 0.07	7.98 ± 0.11	7.63 ± 0.12	7.61 ± 0.12	7.75 ± 0.09
Week 14	9.28 ± 0.10	9.02 ± 0.13	8.95 ± 0.09	9.08 ± 0.11	8.99 ± 0.09	9.11 ± 0.10
Reticulocytes (10^6/μL)						
Day 4	0.62 ± 0.02	0.60 ± 0.02	0.62 ± 0.03	0.59 ± 0.04	0.60 ± 0.03	0.58 ± 0.03
Day 23	0.32 ± 0.01	0.31 ± 0.01	0.31 ± 0.02	0.32 ± 0.02	0.32 ± 0.02	0.32 ± 0.01
Week 14	0.19 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.23 ± 0.01**	0.22 ± 0.01**	0.21 ± 0.01**
Mean cell volume (fL)						
Day 4	61.0 ± 0.3	60.3 ± 0.3	60.4 ± 0.3	61.1 ± 0.3	60.7 ± 0.4	60.6 ± 0.4
Day 23	59.2 ± 0.4	59.4 ± 0.3	59.5 ± 0.3	59.3 ± 0.4	59.3 ± 0.5	59.4 ± 0.4
Week 14	55.6 ± 0.2	55.4 ± 0.2	55.7 ± 0.2	55.4 ± 0.2	55.7 ± 0.2	55.5 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	18.4 ± 0.1	18.5 ± 0.2	18.3 ± 0.1	18.6 ± 0.1	18.5 ± 0.1	18.4 ± 0.1
Day 23	19.7 ± 0.1	20.0 ± 0.1	19.8 ± 0.1	19.8 ± 0.1	19.8 ± 0.2	19.8 ± 0.1
Week 14	16.9 ± 0.1	16.8 ± 0.1	16.7 ± 0.1	16.7 ± 0.1	16.6 ± 0.1	16.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	30.2 ± 0.2	30.6 ± 0.2	30.4 ± 0.2	30.4 ± 0.2	30.4 ± 0.2	30.4 ± 0.2
Day 23	33.2 ± 0.2	33.7 ± 0.2	33.2 ± 0.2	33.4 ± 0.3	33.4 ± 0.2	33.4 ± 0.3
Week 14	31.8 ± 0.2	31.9 ± 0.2	31.3 ± 0.2	31.8 ± 0.2	31.5 ± 0.2	31.7 ± 0.2
Platelets (10^3/μL)						
Day 4	1,070.4 ± 27.0	1,036.2 ± 27.3	1,100.6 ± 31.4	1,036.9 ± 18.3	1,057.6 ± 23.0	1,056.8 ± 28.8
Day 23	780.2 ± 41.4	838.9 ± 23.3	883.6 ± 26.5	864.3 ± 22.5	911.8 ± 18.5**	909.2 ± 19.1**
Week 14	582.8 ± 15.7	568.7 ± 28.4	632.5 ± 19.7	592.1 ± 56.8	659.2 ± 32.4*	713.1 ± 16.0**
Leukocytes (10^3/μL)						
Day 4	8.47 ± 0.38	8.36 ± 0.24	8.64 ± 0.30	8.11 ± 0.42	7.41 ± 0.26	7.79 ± 0.61
Day 23	6.36 ± 0.54	6.27 ± 0.49	6.20 ± 0.32	6.12 ± 0.50	6.03 ± 0.34	5.98 ± 0.47
Week 14	8.30 ± 0.53	7.53 ± 0.52	8.67 ± 0.57	8.43 ± 0.53	7.92 ± 0.63	8.99 ± 0.42
Segmented neutrophils (10^3/μL)						
Day 4	1.15 ± 0.03	1.18 ± 0.08	1.29 ± 0.06	1.07 ± 0.05	1.11 ± 0.07	1.03 ± 0.07
Day 23	0.92 ± 0.11	0.84 ± 0.06	0.86 ± 0.04	0.80 ± 0.05	0.76 ± 0.05	0.88 ± 0.04
Week 14	1.08 ± 0.04	1.18 ± 0.09	1.16 ± 0.06	1.17 ± 0.06	1.06 ± 0.07	1.24 ± 0.08
Lymphocytes (10^3/μL)						
Day 4	7.00 ± 0.36	6.82 ± 0.20	7.03 ± 0.27	6.72 ± 0.38	6.00 ± 0.24	6.47 ± 0.53
Day 23	5.22 ± 0.45	5.22 ± 0.45	5.14 ± 0.30	5.12 ± 0.44	5.09 ± 0.30	4.91 ± 0.43
Week 14	6.91 ± 0.49	6.10 ± 0.51	7.26 ± 0.51	6.98 ± 0.52	6.62 ± 0.62	7.46 ± 0.43
Monocytes (10^3/μL)						
Day 4	0.24 ± 0.01	0.28 ± 0.01	0.24 ± 0.02	0.25 ± 0.02	0.22 ± 0.02	0.23 ± 0.03
Day 23	0.11 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	0.09 ± 0.01
Week 14	0.19 ± 0.02	0.12 ± 0.01*	0.16 ± 0.02	0.17 ± 0.01	0.14 ± 0.01	0.16 ± 0.01

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	9	10	9
Day 23	10	9	10	10	10	9
Week 14	10	10	10	10	9	10
Basophils (10^3/μL)						
Day 4	0.043 ± 0.004	0.035 ± 0.004	0.030 ± 0.004	0.033 ± 0.004	0.034 ± 0.007	0.028 ± 0.006
Day 23	0.029 ± 0.006	0.023 ± 0.003	0.028 ± 0.004	0.022 ± 0.003	0.016 ± 0.002	0.022 ± 0.003
Week 14	0.028 ± 0.005	0.029 ± 0.004	0.028 ± 0.002	0.028 ± 0.005	0.024 ± 0.002	0.035 ± 0.004
Eosinophils (10^3/μL)						
Day 4	0.04 ± 0.00	0.05 ± 0.01	0.05 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.04 ± 0.00
Day 23	0.08 ± 0.02	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.02
Week 14	0.08 ± 0.01	0.10 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.10 ± 0.02
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	10.2 ± 0.5	11.1 ± 0.4	10.4 ± 0.4	9.9 ± 0.4	10.8 ± 0.6	9.9 ± 0.5
Day 23	13.2 ± 0.4	14.9 ± 0.4	14.9 ± 0.6	13.4 ± 0.8	14.0 ± 0.4	14.8 ± 0.7
Week 14	13.0 ± 0.6	13.0 ± 0.4	12.3 ± 0.4	11.9 ± 0.4	12.5 ± 0.5	11.8 ± 0.4
Creatinine (mg/dL)						
Day 4	0.40 ± 0.00	0.40 ± 0.00	0.41 ± 0.01	0.40 ± 0.00	0.40 ± 0.00	0.40 ± 0.00
Day 23	0.47 ± 0.02	0.49 ± 0.01	0.49 ± 0.01	0.49 ± 0.01	0.47 ± 0.02	0.44 ± 0.02
Week 14	0.54 ± 0.02	0.54 ± 0.02	0.51 ± 0.01	0.53 ± 0.02	0.53 ± 0.02	0.52 ± 0.01
Glucose (mg/dL)						
Day 4	139 ± 2	130 ± 2*	133 ± 2	133 ± 2	136 ± 3	136 ± 3
Day 23	147 ± 4	140 ± 4	141 ± 3	144 ± 5	148 ± 4	136 ± 3
Week 14	132 ± 3	138 ± 4	131 ± 4	132 ± 2	142 ± 5	127 ± 2
Total protein (g/dL)						
Day 4	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1
Day 23	6.5 ± 0.0	6.7 ± 0.1**	6.8 ± 0.1**	6.7 ± 0.1**	6.8 ± 0.1**	7.0 ± 0.1**
Week 14	6.6 ± 0.0	6.8 ± 0.1	6.9 ± 0.1**	7.0 ± 0.1**	7.2 ± 0.1**	7.2 ± 0.1**
Albumin (g/dL)						
Day 4	4.0 ± 0.0	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.0	4.0 ± 0.1	4.0 ± 0.1
Day 23	4.4 ± 0.0	4.5 ± 0.0*	4.5 ± 0.0*	4.4 ± 0.0*	4.5 ± 0.1*	4.6 ± 0.0**
Week 14	4.6 ± 0.0	4.7 ± 0.0	4.8 ± 0.0*	4.8 ± 0.0**	4.9 ± 0.0**	4.9 ± 0.0**
Alanine aminotransferase (IU/L)						
Day 4	64 ± 2	66 ± 3	65 ± 2	64 ± 2	64 ± 2	65 ± 2
Day 23	57 ± 1	62 ± 2	64 ± 4	56 ± 1	58 ± 2	58 ± 3
Week 14	74 ± 6	41 ± 1**	45 ± 2**	42 ± 1**	45 ± 1**	42 ± 1**
Alkaline phosphatase (IU/L)						
Day 4	707 ± 11	699 ± 14	678 ± 17	701 ± 14	699 ± 22	697 ± 13
Day 23	482 ± 6	468 ± 12	464 ± 10	451 ± 13*	450 ± 11*	446 ± 7**
Week 14	224 ± 6	210 ± 5	200 ± 3**	188 ± 3**	194 ± 5**	181 ± 3**
Creatine kinase (IU/L)						
Day 4	341 ± 38	462 ± 48	507 ± 44	401 ± 55	427 ± 58	410 ± 54
Day 23	253 ± 19	260 ± 48	268 ± 28	342 ± 37	237 ± 32	334 ± 77
Week 14	149 ± 32	209 ± 26	145 ± 24	321 ± 65	191 ± 82	294 ± 54

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Sorbitol dehydrogenase (IU/L)						
Day 4	15 ± 0	14 ± 1	13 ± 1	16 ± 1	14 ± 1	14 ± 1
Day 23	15 ± 1	16 ± 0	14 ± 1	14 ± 1	13 ± 1	12 ± 0*
Week 14	13 ± 1	10 ± 0*	10 ± 1	11 ± 0	11 ± 1	10 ± 1*
Bile salts (µmol/L)						
Day 4	9.6 ± 1.0	7.3 ± 0.8	5.9 ± 0.8*	7.0 ± 0.8*	5.5 ± 1.2**	5.3 ± 0.5**
Day 23	4.5 ± 0.9	2.7 ± 0.4	3.9 ± 0.6	4.4 ± 0.7	2.7 ± 0.2	2.9 ± 0.4
Week 14	5.5 ± 0.8	3.8 ± 0.4	3.9 ± 0.5	3.3 ± 0.4**	3.8 ± 0.7*	2.8 ± 0.4**
Female						
Hematology						
n						
Day 4	10	10	10	10	10	9
Day 23	10	10	10	10	9	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	45.9 ± 0.6	44.9 ± 0.7	45.3 ± 0.6	45.7 ± 0.9	44.9 ± 0.7	43.7 ± 0.5
Day 23	46.0 ± 0.6	46.1 ± 0.6	46.1 ± 0.7	45.6 ± 0.7	45.1 ± 0.6	45.3 ± 0.4
Week 14	47.5 ± 0.4	47.4 ± 0.4	46.7 ± 0.4	46.6 ± 0.6	45.9 ± 0.5*	46.3 ± 0.5*
Hemoglobin (g/dL)						
Day 4	14.2 ± 0.1	13.9 ± 0.2	14.0 ± 0.1	14.1 ± 0.3	13.8 ± 0.2	13.6 ± 0.2
Day 23	15.4 ± 0.2	15.2 ± 0.1	15.4 ± 0.2	15.1 ± 0.1	14.9 ± 0.1	15.1 ± 0.1
Week 14	15.1 ± 0.1	15.2 ± 0.1	15.0 ± 0.1	14.8 ± 0.1	14.8 ± 0.1	14.9 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	7.68 ± 0.09	7.54 ± 0.11	7.61 ± 0.08	7.65 ± 0.15	7.55 ± 0.13	7.37 ± 0.10
Day 23	7.86 ± 0.08	7.86 ± 0.09	7.90 ± 0.10	7.77 ± 0.11	7.74 ± 0.10	7.75 ± 0.07
Week 14	8.49 ± 0.10	8.50 ± 0.06	8.43 ± 0.06	8.27 ± 0.09	8.37 ± 0.06	8.37 ± 0.06
Reticulocytes (10^6/µL)						
Day 4	0.48 ± 0.02	0.49 ± 0.03	0.48 ± 0.02	0.48 ± 0.03	0.46 ± 0.03	0.46 ± 0.03
Day 23	0.19 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.00
Week 14	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.00	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01
Mean cell volume (fL)						
Day 4	59.7 ± 0.3	59.5 ± 0.3	59.5 ± 0.3	59.8 ± 0.3	59.5 ± 0.3	59.2 ± 0.3
Day 23	58.5 ± 0.3	58.7 ± 0.2	58.3 ± 0.3	58.7 ± 0.3	58.3 ± 0.3	58.4 ± 0.3
Week 14	57.2 ± 0.2	57.5 ± 0.2	57.4 ± 0.2	57.5 ± 0.2	57.2 ± 0.2	57.1 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	18.5 ± 0.1	18.4 ± 0.1	18.4 ± 0.1	18.4 ± 0.1	18.3 ± 0.1	18.5 ± 0.1
Day 23	19.5 ± 0.2	19.4 ± 0.2	19.5 ± 0.2	19.4 ± 0.2	19.3 ± 0.1	19.6 ± 0.1
Week 14	17.8 ± 0.1	17.9 ± 0.1	17.8 ± 0.1	17.8 ± 0.1	17.7 ± 0.1	17.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	30.9 ± 0.2	31.0 ± 0.1	31.0 ± 0.2	30.8 ± 0.2	30.8 ± 0.2	31.2 ± 0.2
Day 23	33.4 ± 0.4	33.1 ± 0.2	33.4 ± 0.4	33.1 ± 0.4	33.2 ± 0.3	33.5 ± 0.2
Week 14	31.9 ± 0.2	32.0 ± 0.1	32.1 ± 0.3	31.7 ± 0.2	32.3 ± 0.2	32.2 ± 0.2

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	10	10	9
Day 24	10	10	10	10	9	10
Week 14	10	10	10	10	10	10
Platelets (10^3/μL)						
Day 4	969.7 ± 28.2	972.7 ± 23.0	1,014.8 ± 29.0	989.7 ± 27.4	989.0 ± 34.6	1,020.9 ± 26.7
Day 23	805.6 ± 13.5	811.1 ± 12.0	749.6 ± 21.1	803.8 ± 19.8	778.7 ± 22.0	803.5 ± 24.4
Week 14	663.1 ± 17.0	614.4 ± 26.4	615.3 ± 29.6	677.3 ± 15.3	662.4 ± 36.9	659.8 ± 30.8
Leukocytes (10^3/μL)						
Day 4	8.22 ± 0.49	8.49 ± 0.29	8.43 ± 0.47	7.29 ± 0.58	7.68 ± 0.28	7.88 ± 0.25
Day 23	7.80 ± 0.55	6.63 ± 0.27	7.03 ± 0.36	6.31 ± 0.46	6.67 ± 0.35	6.42 ± 0.55
Week 14	6.24 ± 0.40	6.31 ± 0.33	5.21 ± 0.51	5.52 ± 0.45	5.62 ± 0.44	6.39 ± 0.38
Segmented neutrophils (10^3/μL)						
Day 4	0.99 ± 0.09	1.07 ± 0.07	1.06 ± 0.05	0.87 ± 0.07	1.11 ± 0.06	0.93 ± 0.05
Day 23	0.79 ± 0.06	0.75 ± 0.05	1.04 ± 0.13	0.76 ± 0.09	0.80 ± 0.07	0.79 ± 0.06
Week 14	0.94 ± 0.07	0.93 ± 0.08	0.88 ± 0.11	1.14 ± 0.09	0.97 ± 0.12	1.09 ± 0.13
Lymphocytes (10^3/μL)						
Day 4	6.94 ± 0.42	7.09 ± 0.26	7.04 ± 0.41	6.15 ± 0.50	6.26 ± 0.24	6.63 ± 0.22
Day 23	6.80 ± 0.49	5.69 ± 0.23	5.78 ± 0.27	5.37 ± 0.39*	5.68 ± 0.27	5.44 ± 0.49
Week 14	5.10 ± 0.32	5.13 ± 0.30	4.13 ± 0.49	4.15 ± 0.38	4.49 ± 0.36	5.09 ± 0.32
Monocytes (10^3/μL)						
Day 4	0.20 ± 0.02	0.23 ± 0.01	0.23 ± 0.02	0.19 ± 0.02	0.22 ± 0.01	0.22 ± 0.01
Day 23	0.13 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.13 ± 0.02	0.11 ± 0.02
Week 14	0.11 ± 0.01	0.14 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.12 ± 0.02
Basophils (10^3/μL)						
Day 4	0.032 ± 0.003	0.036 ± 0.006	0.039 ± 0.007	0.029 ± 0.005	0.037 ± 0.007	0.037 ± 0.004
Day 23	0.039 ± 0.010	0.022 ± 0.004	0.027 ± 0.006	0.014 ± 0.002	0.016 ± 0.002	0.022 ± 0.005
Week 14	0.018 ± 0.002	0.015 ± 0.003	0.019 ± 0.004	0.017 ± 0.002	0.014 ± 0.003	0.018 ± 0.002
Eosinophils (10^3/μL)						
Day 4	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.00
Day 23	0.05 ± 0.00	0.04 ± 0.00	0.06 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
Week 14	0.08 ± 0.01	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.02	0.06 ± 0.01	0.08 ± 0.02
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	10.9 ± 0.5	10.2 ± 0.5	11.9 ± 0.4	11.5 ± 0.8	10.5 ± 0.6	10.8 ± 0.6
Day 23	12.3 ± 0.3	13.6 ± 0.6	12.4 ± 0.7	12.5 ± 0.5	12.8 ± 0.7	12.6 ± 0.4
Week 14	12.7 ± 0.5	13.5 ± 0.4	13.3 ± 0.8	12.1 ± 0.4	11.6 ± 0.5	12.6 ± 0.4
Creatinine (mg/dL)						
Day 4	0.41 ± 0.01	0.39 ± 0.01	0.41 ± 0.01	0.40 ± 0.00	0.40 ± 0.00	0.40 ± 0.00
Day 23	0.46 ± 0.02	0.50 ± 0.00	0.48 ± 0.01	0.50 ± 0.00	0.48 ± 0.01	0.48 ± 0.01
Week 14	0.56 ± 0.02	0.58 ± 0.03	0.57 ± 0.02	0.54 ± 0.02	0.52 ± 0.01	0.57 ± 0.02
Glucose (mg/dL)						
Day 4	144 ± 5	137 ± 4	138 ± 6	146 ± 5	135 ± 3	135 ± 3
Day 23	140 ± 4	145 ± 6	133 ± 3	145 ± 6	143 ± 4	137 ± 5
Week 14	117 ± 3	123 ± 4	125 ± 2	129 ± 6	130 ± 6	121 ± 4
Total protein (g/dL)						
Day 4	6.0 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	5.9 ± 0.1
Day 23	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.4 ± 0.1
Week 14	7.0 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.4 ± 0.1

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Female (continued)						
Clinical chemistry (continued)						
n	10	10	10	10	10	10
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.2 ± 0.0	4.3 ± 0.0	4.4 ± 0.0	4.2 ± 0.1	4.2 ± 0.0
Day 23	4.4 ± 0.1	4.4 ± 0.0	4.4 ± 0.1	4.4 ± 0.0	4.3 ± 0.0	4.4 ± 0.0
Week 14	5.1 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.1 ± 0.1	5.1 ± 0.1	5.1 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	54 ± 2	54 ± 2	53 ± 2	57 ± 2	53 ± 2	52 ± 2
Day 23	40 ± 1	44 ± 1*	41 ± 1	43 ± 1	40 ± 1	41 ± 2
Week 14	49 ± 3	45 ± 1	40 ± 1**	39 ± 1**	39 ± 1**	35 ± 1**
Alkaline phosphatase (IU/L)						
Day 4	563 ± 10	536 ± 19	518 ± 10	546 ± 14	517 ± 18	519 ± 20
Day 23	365 ± 5	348 ± 11*	340 ± 9*	339 ± 8*	320 ± 8**	311 ± 9**
Week 14	183 ± 7	160 ± 7*	159 ± 4*	138 ± 4**	146 ± 4**	135 ± 3**
Creatine kinase (IU/L)						
Day 4	380 ± 43	331 ± 47	321 ± 41	309 ± 42	307 ± 33	368 ± 60
Day 23	246 ± 28	241 ± 25	237 ± 57	257 ± 50	207 ± 29	336 ± 85
Week 14	258 ± 54	211 ± 30	165 ± 31	201 ± 27	160 ± 29	181 ± 21
Sorbitol dehydrogenase (IU/L)						
Day 4	13 ± 1	13 ± 1	14 ± 0	15 ± 0*	13 ± 1	14 ± 1
Day 23	12 ± 1	12 ± 1	11 ± 1	12 ± 1	13 ± 1	13 ± 1
Week 14	13 ± 1	13 ± 1	12 ± 1	11 ± 1	10 ± 1	11 ± 1
Bile salts (µmol/L)						
Day 4	6.1 ± 1.1	5.8 ± 0.8	3.7 ± 0.4	5.0 ± 0.7	3.6 ± 0.4	3.5 ± 0.4
Day 23	5.6 ± 0.9	6.4 ± 0.9	4.9 ± 1.3	5.0 ± 0.8	5.3 ± 1.0	3.3 ± 0.5
Week 14	10.0 ± 1.2	8.7 ± 0.9	6.0 ± 0.9*	4.3 ± 0.5**	3.4 ± 0.5**	3.1 ± 0.4**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

TABLE F2
Thyroid Hormone Data for Rats in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Male				
n	9	10	10	10
Thyroid stimulating hormone (ng/mL)				
Day 22	8.33 ± 0.47	8.38 ± 0.89[b]	10.78 ± 1.49[c]	11.20 ± 0.96
Week 14	6.89 ± 0.56	9.10 ± 0.50**	9.60 ± 0.75**	10.90 ± 0.81**
Triiodothyronine (ng/dL)				
Day 22	154.6 ± 5.6	167.9 ± 8.2	149.8 ± 6.9	142.5 ± 10.2
Week 14	128.7 ± 6.8	145.4 ± 7.6	132.2 ± 5.2	143.9 ± 6.0
Total thyroxine (ng/dL)				
Day 22	5.78 ± 0.23	6.51 ± 0.28	6.39 ± 0.15	5.63 ± 0.33
Week 14	5.23 ± 0.29	5.60 ± 0.22	5.31 ± 0.30	5.88 ± 0.14
Female				
n	10	10	10	10
Thyroid-stimulating hormone (ng/mL)				
Day 22	9.43 ± 0.48[d]	10.00 ± 0.82[c]	9.56 ± 0.53[c]	10.44 ± 0.56[c]
Week 14	5.56 ± 0.34[c]	5.70 ± 0.33	6.40 ± 0.54	7.30 ± 0.40**
Triiodothyronine (mg/dL)				
Day 22	124.7 ± 5.4	130.7 ± 8.0	127.7 ± 5.9	136.7 ± 6.5
Week 14	134.0 ± 6.0	148.9 ± 8.0	155.9 ± 6.1	151.3 ± 8.6
Total thyroxine (ng/dL)				
Day 22	4.19 ± 0.30	4.53 ± 0.28	4.86 ± 0.31	4.64 ± 0.32
Week 14	3.88 ± 0.29	3.31 ± 0.25	4.07 ± 0.28	3.48 ± 0.21

** Significantly different ($P \leq 0.01$) from the vehicle control group by Shirley's test
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=8
[c] n=9
[d] n=7

TABLE F3
Hematology Data for Mice in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Male						
n	10	10	10	10	10	10
Hematocrit (%)	45.9 ± 0.4	47.0 ± 0.5	47.1 ± 0.4	47.0 ± 0.4	46.5 ± 0.4	46.9 ± 0.5
Hemoglobin (g/dL)	14.7 ± 0.1	15.0 ± 0.2	15.1 ± 0.1	15.2 ± 0.1	15.0 ± 0.1	15.3 ± 0.2*
Erythrocytes (10^6/µL)	9.90 ± 0.11	10.04± 0.11	10.05 ± 0.08	10.03 ± 0.09	9.97 ± 0.11	10.08 ± 0.14
Reticulocytes (10^6/µL)	0.27 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.23 ± 0.01**	0.21 ± 0.00**	0.16 ± 0.01**
Mean cell volume (fL)	46.3 ± 0.2	46.8 ± 0.2	46.9 ± 0.3	46.9 ± 0.2	46.7 ± 0.2	46.5 ± 0.2
Mean cell hemoglobin (pg)	14.9 ± 0.1	14.9 ± 0.0	15.0 ± 0.1	15.2 ± 0.1	15.0 ± 0.1	15.2 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.1 ± 0.1	31.9 ± 0.1	32.1 ± 0.2	32.3 ± 0.1	32.2 ± 0.1	32.6 ± 0.2
Platelets (10^3/µL)	1,142.9 ± 40.1	1,168.3 ± 41.1	1,231.0 ± 32.8	1,274.1 ± 49.6*	1,305.3 ± 48.5**	1,375.8 ± 72.4**
Leukocytes (10^3/µL)	4.23 ± 0.73	3.50 ± 0.57	5.12 ± 0.39	5.07 ± 0.30	3.70 ± 0.35	3.98 ± 0.38
Segmented neutrophils (10^3/µL)	0.77 ± 0.12	0.67 ± 0.20	0.89 ± 0.10	0.88 ± 0.10	0.60 ± 0.07	0.62 ± 0.08
Lymphocytes (10^3/µL)	3.28 ± 0.58	2.70 ± 0.40	4.01 ± 0.31	4.02 ± 0.29	2.94 ± 0.29	3.20 ± 0.30
Monocytes (10^3/µL)	0.09 ± 0.02	0.05 ± 0.01	0.11 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
Basophils (10^3/µL)	0.006 ± 0.002	0.009 ± 0.002	0.009 ± 0.002	0.009 ± 0.002	0.005 ± 0.002	0.008 ± 0.001
Eosinophils (10^3/µL)	0.09 ± 0.02	0.08 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.09 ± 0.01
Female						
n	10	10	10	10	9	10
Hematocrit (%)	46.7 ± 0.4	46.0 ± 1.3	46.3 ± 0.6	46.4 ± 0.6	46.1 ± 0.3	47.3 ± 0.4
Hemoglobin (g/dL)	15.4 ± 0.1	15.1 ± 0.4	15.4 ± 0.2	15.5 ± 0.2	15.4 ± 0.1	15.6 ± 0.1
Erythrocytes (10^6/µL)	10.19 ± 0.09	9.90 ± 0.29	10.02 ± 0.12	10.05 ± 0.12	10.11 ± 0.09	10.29 ± 0.07
Reticulocytes (10^6/µL)	0.29 ± 0.01	0.32 ± 0.03	0.29 ± 0.02	0.23 ± 0.01*	0.21 ± 0.01**	0.22 ± 0.01**
Mean cell volume (fL)	45.8 ± 0.2	46.5 ± 0.2	46.2 ± 0.1	46.2 ± 0.2	45.7 ± 0.2	45.9 ± 0.1
Mean cell hemoglobin (pg)	15.1 ± 0.1	15.3 ± 0.1	15.4 ± 0.1	15.4 ± 0.1	15.2 ± 0.1	15.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.1	32.9 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.3 ± 0.2	33.0 ± 0.2
Platelets (10^3/µL)	951.5 ± 48.5	974.0 ± 68.7	977.9 ± 71.2	1,010.6 ± 82.2	1,082.8 ± 46.8	1,133.6 ± 52.5
Leukocytes (10^3/µL)	4.85 ± 0.31	4.66 ± 0.30	4.40 ± 0.34	3.43 ± 0.40*	2.58 ± 0.26**	3.77 ± 0.32**
Segmented neutrophils (10^3/µL)	0.49 ± 0.05	0.40 ± 0.06	0.46 ± 0.05	0.29 ± 0.03*	0.26 ± 0.02**	0.41 ± 0.06
Lymphocytes (10^3/µL)	4.14 ± 0.26	4.05 ± 0.26	3.75 ± 0.29	2.99 ± 0.36*	2.24 ± 0.23**	3.26 ± 0.27**
Monocytes (10^3/µL)	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.03 ± 0.01**	0.05 ± 0.01**
Basophils (10^3/µL)	0.010 ± 0.001	0.007 ± 0.002	0.006 ± 0.002	0.003 ± 0.002*	0.003 ± 0.002	0.006 ± 0.002
Eosinophils (10^3/µL)	0.13 ± 0.02	0.12 ± 0.02	0.10 ± 0.01	0.08 ± 0.01	0.05 ± 0.01**	0.05 ± 0.01**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

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

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	322 ± 5	329 ± 5	322 ± 3	326 ± 4	324 ± 2	314 ± 5
Heart						
Absolute	0.912 ± 0.016	0.911 ± 0.018	0.903 ± 0.016	0.930 ± 0.013	0.939 ± 0.020	0.915 ± 0.023
Relative	2.835 ± 0.022	2.770 ± 0.033	2.805 ± 0.030	2.849 ± 0.028	2.901 ± 0.057	2.908 ± 0.034
R. Kidney						
Absolute	0.987 ± 0.025	0.977 ± 0.022	0.973 ± 0.014	0.999 ± 0.020	1.015 ± 0.012	1.031 ± 0.024
Relative	3.067 ± 0.053	2.973 ± 0.061	3.025 ± 0.043	3.061 ± 0.037	3.137 ± 0.044	3.279 ± 0.046**
Liver						
Absolute	11.296 ± 0.257	13.654 ± 0.300**	13.856 ± 0.357**	14.317 ± 0.244**	15.151 ± 0.219**	15.341 ± 0.511**
Relative	35.106 ± 0.524	41.503 ± 0.634**	43.012 ± 0.710**	43.906 ± 0.828**	46.815 ± 0.565**	48.700 ± 0.982**
Lung						
Absolute	1.974 ± 0.057	1.969 ± 0.086	1.730 ± 0.049	1.884 ± 0.085	1.833 ± 0.081	1.927 ± 0.070
Relative	6.148 ± 0.193	5.995 ± 0.275	5.374 ± 0.133	5.761 ± 0.220	5.667 ± 0.255	6.149 ± 0.260
R. Testis						
Absolute	1.384 ± 0.015	1.402 ± 0.025	1.406 ± 0.017	1.408 ± 0.015	1.408 ± 0.012	1.410 ± 0.016
Relative	4.306 ± 0.041	4.265 ± 0.067	4.375 ± 0.065	4.319 ± 0.055	4.353 ± 0.040	4.494 ± 0.081
Thymus						
Absolute	0.306 ± 0.010	0.287 ± 0.007	0.295 ± 0.014	0.290 ± 0.012	0.288 ± 0.017	0.291 ± 0.015
Relative	0.956 ± 0.042	0.873 ± 0.025	0.920 ± 0.048	0.889 ± 0.041	0.891 ± 0.053	0.927 ± 0.050
Female						
Necropsy body wt	192 ± 2	191 ± 2	194 ± 3	194 ± 4	193 ± 1	193 ± 2
Heart						
Absolute	0.605 ± 0.008	0.604 ± 0.009	0.622 ± 0.013	0.606 ± 0.011	0.618 ± 0.011	0.645 ± 0.020
Relative	3.146 ± 0.037	3.166 ± 0.042	3.218 ± 0.066	3.137 ± 0.072	3.211 ± 0.057	3.334 ± 0.086
R. Kidney						
Absolute	0.664 ± 0.010	0.655 ± 0.008	0.649 ± 0.014	0.663 ± 0.015	0.679 ± 0.015	0.680 ± 0.016
Relative	3.454 ± 0.059	3.437 ± 0.046	3.356 ± 0.063	3.426 ± 0.058	3.528 ± 0.075	3.521 ± 0.077
Liver						
Absolute	5.952 ± 0.110	6.590 ± 0.134**	6.548 ± 0.132**	7.286 ± 0.208**	7.485 ± 0.098**	8.026 ± 0.128**
Relative	30.949 ± 0.497	34.521 ± 0.375**	33.841 ± 0.552**	37.576 ± 0.595**	38.905 ± 0.614**	41.547 ± 0.631**
Lung						
Absolute	1.173 ± 0.039	1.309 ± 0.114	1.240 ± 0.032	1.164 ± 0.037	1.267 ± 0.077	1.254 ± 0.059
Relative	6.104 ± 0.212	6.844 ± 0.549	6.422 ± 0.198	6.014 ± 0.184	6.586 ± 0.412	6.479 ± 0.273
Thymus						
Absolute	0.242 ± 0.012	0.244 ± 0.008	0.246 ± 0.007	0.249 ± 0.011	0.254 ± 0.015	0.247 ± 0.005
Relative	1.260 ± 0.060	1.281 ± 0.038	1.269 ± 0.019	1.282 ± 0.047	1.317 ± 0.075	1.279 ± 0.027

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

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

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at 14 Weeks in the 2-Year Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Male				
n	9	10	10	10
Necropsy body weight	335 ± 9	329 ± 5	330 ± 4	328 ± 3
Liver				
Absolute	12.24 ± 0.38	14.22 ± 0.29**	15.07 ± 0.23**	16.09 ± 0.25**
Relative	36.589 ± 0.634	43.178 ± 0.746**	45.650 ± 0.647**	49.087 ± 0.591**
Thyroid gland				
Absolute	0.026 ± 0.001	0.029 ± 0.001	0.027 ± 0.001	0.028 ± 0.002
Relative	0.076 ± 0.004	0.089 ± 0.002*	0.083 ± 0.003	0.087 ± 0.005
Female				
n	10	10	10	10
Necropsy body weight	194 ± 4	200 ± 3	193 ± 3	198 ± 3
Liver				
Absolute	6.37 ± 0.20	7.03 ± 0.14**	7.28 ± 0.15**	8.60 ± 0.16**
Relative	32.812 ± 0.643	35.092 ± 0.585**	37.703 ± 0.481**	43.345 ± 0.427**
Thyroid gland				
Absolute	0.023 ± 0.001	0.026 ± 0.001	0.023 ± 0.001	0.025 ± 0.001
Relative	0.119 ± 0.004	0.128 ± 0.006	0.120 ± 0.007	0.127 ± 0.006

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

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	33.6 ± 0.9	34.4 ± 0.6	34.3 ± 0.8	34.0 ± 1.0	33.2 ± 0.3	32.4 ± 0.6
Heart						
Absolute	0.185 ± 0.011	0.183 ± 0.007	0.179 ± 0.005	0.171 ± 0.007	0.169 ± 0.007	0.175 ± 0.009
Relative	5.516 ± 0.308	5.323 ± 0.193	5.262 ± 0.230	5.063 ± 0.231	5.082 ± 0.211	5.404 ± 0.314
R. Kidney						
Absolute	0.275 ± 0.006	0.270 ± 0.004	0.271 ± 0.003	0.268 ± 0.008	0.258 ± 0.006	0.244 ± 0.008**
Relative	8.233 ± 0.187	7.864 ± 0.168	7.957 ± 0.209	7.888 ± 0.219	7.772 ± 0.190	7.524 ± 0.161*
Liver						
Absolute	1.595 ± 0.045	1.747 ± 0.025	1.902 ± 0.043**	1.996 ± 0.086**	2.169 ± 0.049**	2.453 ± 0.066**
Relative	47.583 ± 0.758	50.796 ± 0.647*	55.519 ± 0.670**	58.511 ± 0.952**	65.340 ± 0.150**	75.593 ± 1.111**
Lung						
Absolute	0.230 ± 0.011	0.248 ± 0.015	0.228 ± 0.007	0.234 ± 0.012	0.209 ± 0.010	0.231 ± 0.011
Relative	6.878 ± 0.312	7.217 ± 0.436	6.711 ± 0.299	6.941 ± 0.401	6.303 ± 0.281	7.116 ± 0.316
R. Testis						
Absolute	0.117 ± 0.003	0.116 ± 0.002	0.119 ± 0.002	0.119 ± 0.003	0.119 ± 0.002	0.119 ± 0.003
Relative	3.528 ± 0.089	3.368 ± 0.084	3.487 ± 0.088	3.501 ± 0.113	3.586 ± 0.077	3.658 ± 0.051
Thymus						
Absolute	0.043 ± 0.003	0.042 ± 0.003	0.041 ± 0.002	0.042 ± 0.001	0.045 ± 0.002	0.040 ± 0.003
Relative	1.272 ± 0.098	1.228 ± 0.088	1.207 ± 0.059	1.239 ± 0.056	1.347 ± 0.068	1.245 ± 0.079
Female						
n	10	10	10	10	9	10
Necropsy body wt	27.8 ± 0.3	27.9 ± 1.1	27.4 ± 0.5	27.3 ± 0.6	26.1 ± 0.5	25.4 ± 0.2*
Heart						
Absolute	0.147 ± 0.008	0.149 ± 0.010	0.139 ± 0.005	0.144 ± 0.007	0.147 ± 0.007	0.139 ± 0.007
Relative	5.311 ± 0.279	5.374 ± 0.337	5.093 ± 0.186	5.301 ± 0.311	5.649 ± 0.276	5.464 ± 0.250
R. Kidney						
Absolute	0.160 ± 0.004	0.158 ± 0.004	0.162 ± 0.004	0.155 ± 0.006	0.153 ± 0.004	0.151 ± 0.003
Relative	5.752 ± 0.162	5.688 ± 0.152	5.907 ± 0.152	5.697 ± 0.210	5.879 ± 0.171	5.940 ± 0.105
Liver						
Absolute	1.108 ± 0.025	1.236 ± 0.045**	1.275 ± 0.027**	1.322 ± 0.039**	1.390 ± 0.026**	1.663 ± 0.030**
Relative	39.965 ± 1.048	44.429 ± 0.980**	46.533 ± 0.762**	48.409 ± 0.799**	53.252 ± 1.103**	65.362 ± 0.906**
Lung						
Absolute	0.261 ± 0.013	0.259 ± 0.023	0.252 ± 0.016	0.258 ± 0.019	0.237 ± 0.014	0.228 ± 0.014
Relative	9.424 ± 0.458	9.276 ± 0.693	9.199 ± 0.536	9.418 ± 0.599	9.104 ± 0.589	8.960 ± 0.510
Thymus						
Absolute	0.052 ± 0.003	0.055 ± 0.003	0.056 ± 0.002	0.052 ± 0.003	0.051 ± 0.002	0.047 ± 0.002
Relative	1.860 ± 0.131	1.964 ± 0.102	2.019 ± 0.068	1.889 ± 0.097	1.967 ± 0.081	1.836 ± 0.083

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

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	322 ± 5	326 ± 4	324 ± 2	314 ± 5
L. Cauda epididymis	0.1360 ± 0.0029	0.1283 ± 0.0042	0.1297 ± 0.0071	0.1326 ± 0.0028
L. Epididymis	0.4008 ± 0.0050	0.3997 ± 0.0065	0.4034 ± 0.0078	0.4055 ± 0.0068
L. Testis	1.4167 ± 0.0218	1.4656 ± 0.0166	1.4777 ± 0.0116	1.4635 ± 0.0211
Spermatid measurements				
Spermatid heads (10^6/testis)	170.3 ± 5.7	162.9 ± 2.5	174.5 ± 3.1	176.1 ± 4.9
Spermatid heads (10^6/g testis)	136.6 ± 4.7	121.1 ± 1.8*	133.1 ± 2.8	128.0 ± 3.5
Epididymal spermatozoal measurements				
Sperm motility (%)	85.60 ± 0.81	85.90 ± 0.85	77.60 ± 7.55	85.00 ± 0.88
Sperm (10^6/cauda epididymis)	92.4 ± 5.9	91.3 ± 3.8	74.5 ± 7.9	89.9 ± 5.9
Sperm (10^6/g cauda epididymis)	680 ± 42	713 ± 24	562 ± 49	679 ± 43

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

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid heads per testis and epididymal spermatozoal measurements).

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	192 ± 2	194 ± 4	193 ± 1	193 ± 2
Proportion of regular cycling females[b]	10/10	10/10	10/10	10/10
Estrous cycle length (days)	5.00 ± 0.15	5.20 ± 0.13	5.05 ± 0.05	4.95 ± 0.05
Estrous stages (% of cycle)				
Diestrus	60.0	64.2	65.8	60.8
Proestrus	15.0	14.2	12.5	15.0
Estrus	20.8	20.0	19.2	20.8
Metestrus	4.2	0.8	0.8	1.7
Uncertain diagnosis	0.0	0.8	1.7	1.7

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices indicated no significant differences in the probability of an altered cycle for any treated group compared to the vehicle controls.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	500 mg/kg	1,000 mg/kg	2,000 mg/kg
n	9	10	10	10
Weights (g)				
Necropsy body wt	33.4 ± 1.0	34.0 ± 1.0	33.2 ± 0.3	32.4 ± 0.6
L. Cauda epididymis	0.0153 ± 0.0014	0.0141 ± 0.0006	0.0154 ± 0.0008	0.0144 ± 0.0006
L. Epididymis	0.0423 ± 0.0020	0.0438 ± 0.0008	0.0437 ± 0.0010	0.0408 ± 0.0014
L. Testis	0.1121 ± 0.0019	0.1161 ± 0.0024	0.1116 ± 0.0019	0.1128 ± 0.0023
Spermatid measurements				
Spermatid heads (10^6/testis)	17.89 ± 0.72	17.52 ± 0.87	18.63 ± 1.00	18.74 ± 0.91
Spermatid heads (10^6/g testis)	205.5 ± 8.9	194.7 ± 9.8	215.9 ± 9.4	218.6 ± 8.0
Epididymal spermatozoal measurements				
Sperm motility (%)	87.22 ± 0.36	87.70 ± 0.73	87.30 ± 0.56	87.10 ± 0.48
Sperm (10^6/cauda epididymis)	14.0 ± 1.5	15.5 ± 1.7	16.1 ± 1.4	16.0 ± 1.7
Sperm (10^6/g cauda epididymis)	931 ± 85	1,121 ± 129	1,029 ± 52	1,106 ± 108

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of *Ginkgo biloba* Extract[a]

	Vehicle Control	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Number weighed at necropsy	10	10	9	10
Necropsy body wt (g)	27.8 ± 0.3	27.3 ± 0.6	26.1 ± 0.5*	25.4 ± 0.2**
Proportion of regular cycling females[b]	6/8	8/10	7/9	5/10
Estrous cycle length (days)	4.04 ± 0.13[c]	4.97 ± 0.47	4.83 ± 0.31	4.67 ± 0.40[d]
Estrous stages (% of cycle)				
Diestrus	41.7	40.8	31.5	40.8
Proestrus	0.0	0.0	0.0	0.0
Estrus	40.8	36.7	43.5	40.0
Metestrus	16.7	22.5	24.1	19.2
Uncertain diagnosis	0.8	0.0	0.9	0.0

* Significantly different ($P \leq 0.05$) from the vehicle control group by William's test
** $P \leq 0.01$
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices indicated a significantly higher probability of extended estrus for female mice in the 2,000 mg/kg group compared to the vehicle controls.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.
[d] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Ginkgo biloba Extract

Although the original study planned to use standardized extract EGB 761®, manufactured by Wilhelm Schwabe, this material was not available to the NTP because at the time of procurement for the NTP studies, this standardized extract was sold unformulated only to Pharma. Through industry contacts, the NTP learned that Shanghai Xing Ling Science and Technology Pharmaceutical Company (Shanghai, China) produced an extract similar to the Schwabe extract and that was widely distributed in commerce. *Ginkgo biloba* extract was obtained from Shanghai Xing Ling Science and Technology Pharmaceutical Company, Ltd. in two lots (020703 and GBE-50-001003). Lot 020703 was used during the 3-month and 2-year studies. Identity, purity, stability, and moisture analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO); in addition, the study laboratory at Battelle Columbus Operations (Columbus, OH) confirmed the identity of the test article versus a frozen reference of the same lot, shipped separately, by infrared spectroscopy. Reports on analyses performed in support of the *Ginkgo biloba* extract studies are on file at the National Institute of Environmental Health Sciences.

The identity and purity of the test article were determined by using a combination of techniques and authentic standards specific for ginkgo. Estimation of the distribution of particle sizes in lot GBE-50-001003 was determined using a sieve method, and the results indicated that 84.3% of the test article passed through a No. 140 (106 µm) sieve (Table I1). The bulk density of lot 020703 was determined to be 0.521 g/mL. To evaluate organic constituents of the test article, extracts were prepared, and a combination of chromatographic and spectrometric techniques was used to characterize this lot. High-performance thin layer chromatography (HPTLC) fingerprint analyses were conducted for ginkgolides and flavonoids in methanol extracts of the test article using two systems specified in Application Notes F-16A and F-16B, respectively, obtained from CAMAG Scientific, Inc. (Wilmington, NC). Many components of the test article were tentatively identified using various chromatography/mass spectrometry techniques, including liquid chromatography/mass spectrometry, gas chromatography/mass spectrometry, and matrix-assisted laser-desorption ionization time-of-flight mass spectrometry. High-performance liquid chromatography (HPLC) was used to profile ethanol:water:12N hydrochloric acid (64:26:10) extracts of the bulk material and to quantitate (using caffeine as an internal standard) α-glycosides and terpenoids in these extracts. Analyses were conducted on nonhydrolyzed extracts and also on extracts hydrolyzed at 90° C for at least 2 hours, utilizing commercially obtained standards with matching retention times published in the literature (Hasler *et al*., 1992). The HPLC system used a Spectra SYSTEM™ liquid chromatograph (Thermo Fisher Scientific, Inc., Pittsburgh, PA), ultraviolet (UV) and evaporative light scattering (ELS) detectors (Applied Biosystems, Foster City, CA, and SEDERE, Inc., Cranbury, NJ, respectively) and a Prodigy® ODS3 5 µm column (250 mm × 4.6 mm; Phenomenex, Torrance, CA) maintained at ambient temperature. For these assays, a mobile phase of A: water:methanol (90:10) containing 0.25% formic acid and B: methanol containing 0.25% formic acid was linearly ramped; from 15% B to 38% B in 23 minutes, then to 45% B in 2 minutes with a 30-minute hold, and then to 15% B in 5 minutes with a 5 minute hold. In addition, UV absorbance was recorded at 270 nm, and the flow rate of the mobile phase was 1.0 mL/minute. For the ginkgolic acids and colchicines analyses, the HPLC system consisted of a Hewlett-Packard 1090 Series II chromatograph (Palo Alto, CA), UV detector [Thermo Separation Products Spectromonitor 3200 (Waltham, MA)], and Fisons Instruments Quattro I Mass Spectrometer (St. Louis, MO). For these assays, methanol:water (50:50) extracts of the *Ginkgo biloba* powder were partitioned with dichloromethane and dried over anhydrous sodium sulfate. The residue was reconstituted with methanol and analyzed using total ion current and single ion response mode following the methodology of Ndjoko *et al*. (2000) and Li *et al*. (2002). Further information on these methods can be found in Gray *et al*. (2005, 2007).

As determined by weight loss on drying, moisture content for lot 020703 was 0.90%. Developed, dried HPTLC plates were viewed under UV light, and multiple bands were obtained for the test article by both systems; bands consistent with those for standards obtained from Sigma-Aldrich (St. Louis, MO) of the ginkgolides bilobalide, ginkgolide A, and ginkgolide B, and of the flavonoid rutin hydrate were observed. In HPLC/UV profiles of the test article extracts, 37 components were observed to have peak areas greater than or equal to 0.05% of the total peak area, and three components were identified: quercetin, kaempferol, and isorhamnetin, which had peak areas equal to

34.08%, 27.77%, and 5.43%, respectively, of the total peak area. In HPLC/ELS profiles of the test article extracts, 18 components were observed to have peak areas greater than or equal to 0.05% of the total peak area, and seven components were identified: bilobalide, ginkgolide C, ginkgolide A, ginkgolide B, quercetin, kaempferol, and isorhamnetin, which had peak areas equal to 17.30%, 3.25%, 9.06%, 2.05%, 28.74%, 12.58%, and 2.24%, respectively, of the total peak area. Quantitation assays of α-glycosides in the hydrolyzed extracts using HPLC/UV indicated that the test material contained 16.71% quercetin, 12.20% kaempferol, and 2.37% isorhamnetin. HPLC/ELS quantitation assays of terpenoids in the hydrolyzed extracts determined that the test material contained 6.94% bilobalide, 3.06% ginkgolide C, 3.74% ginkgolide A, and 1.62% ginkgolide B. Representative HPLC chromatograms for the α-glycosides and terpenoids quantitated in these assays are presented in Figures I1 and I2, respectively. HPLC/MS analyses for the presence of ginkgolic acids I, II, and III using standards from ChromaDex, Inc. (Irvine, CA), and for colchicine using the colchicine standard from Sigma-Aldrich, resulted in no observable peaks of ginkgolic acids or colchicine in the test material.

Stability studies of lot 020703 of the bulk chemical were performed using HPLC analyses by the same UV and ELS detection techniques used to characterize this lot of the bulk product. Results of these assays were inconclusive due to high amounts of variability in the measurements, but the seven α-glycosides and terpenoid components in lot 020703 appeared to be stable, within experimental error, at temperatures up to approximately 60° C when the bulk material was stored protected from light. To ensure stability, the bulk chemical was stored at room temperature in amber glass bottles. Periodic reanalyses of the bulk chemical were performed during the 3-month and 2-year studies by the study laboratory using the same HPLC/UV system used to characterize the test material except that the isocratic mobile phase consisted of 0.5% aqueous formic acid:methanol (1:1) and the analytical column was heated to 35° C; no degradation of the bulk material was observed over the course of the studies.

Corn Oil

NF-grade corn oil was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA) and was used as the vehicle in the 3-month and 2-year studies. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than the rejection level of 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing *Ginkgo biloba* extract with corn oil to give the required concentrations (Table I2). The dose formulations were stored at room temperature in sealed plastic bottles enclosed in amber plastic bags for up to 35 days (3-month studies) or 41 days (2-year studies).

Homogeneity studies of the 25 and 400 mg/mL dose formulations were performed by the study laboratory with the isocratic HPLC/UV system previously described for the periodic bulk chemical reanalyses; this system was also used by Battelle Chemistry Support Services (Columbus, OH) to perform stability studies of a 1 mg/mL formulation made with lot GBE-50-001003 of *Ginkgo biloba* extract. Homogeneity was confirmed, and stability was confirmed for at least 42 days for formulations stored in sealed plastic bottles protected from light at room temperature or at approximately 5° C, and for at least 3 hours under simulated animal room conditions.

The study laboratory determined that the 400 mg/mL dose formulation of *Ginkgo biloba* extract in corn oil was gavagable using a 20-gauge gavage needle.

Periodic analyses of the dose formulations of *Ginkgo biloba* extract were conducted by the study laboratory using the isocratic HPLC/UV system previously described and an authentic standard of quercetin dihydrate. During the 3-month studies, the dose formulations were analyzed three times; all 15 dose formulations for rats and mice were within 10% of the target concentrations (Table I3). Animal room samples of these dose formulations were also analyzed; all 15 animal room samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 12 weeks; animal room samples were also analyzed (Table I4). All 30 dose formulations analyzed for rats and mice were within 10% of the target concentrations; all 12 animal room samples for rats and all 12 for mice were within 10% of the target concentrations.

TABLE I1
Particle Size Distribution in *Ginkgo biloba* Extract

U.S. Sieve Number	Sieve Size (µm)	Weight of Powder (g)	Percent of Total	Percent Through
40	425	0.0	0.0	100.0
60	250	0.6	0.6	99.4
70	212	1.1	1.1	98.3
80	180	1.8	1.8	96.5
100	150	2.9	2.9	93.6
120	125	5.0	5.0	88.6
140	106	4.3	4.3	84.3
200	75	12.7	12.6	71.7
270	53	60.4	59.9	11.8
Bottom pan	NA	12.1	12.0	NA

NA = not applicable; the material cannot go through the bottom pan

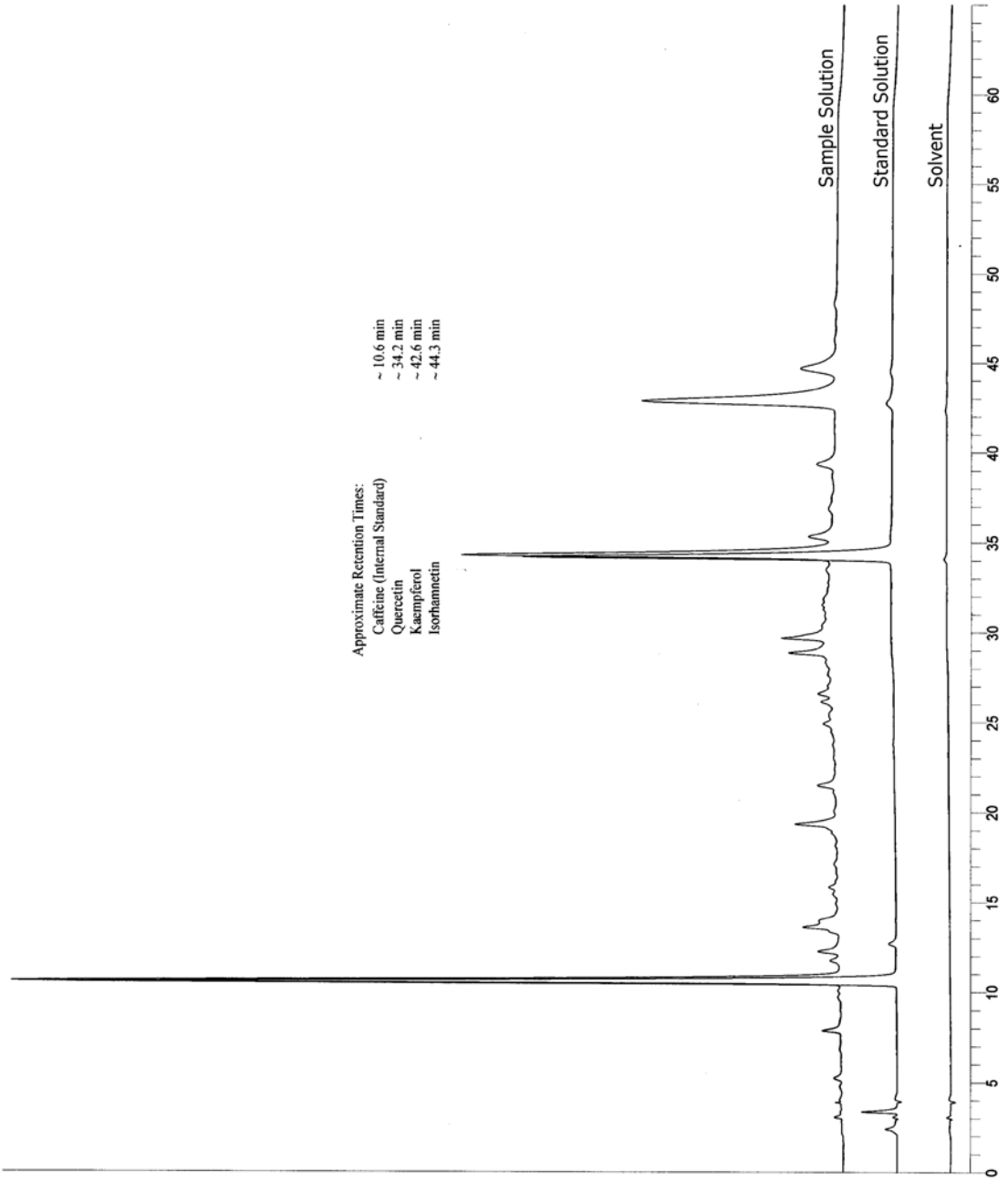

FIGURE I1
High-Performance Liquid Chromatography with Ultraviolet Detection Quantitation of α-Glycosides in *Ginkgo biloba* Extract
Kaempferol and isorhamnetin were determined against the quercetin dihydrate standard.

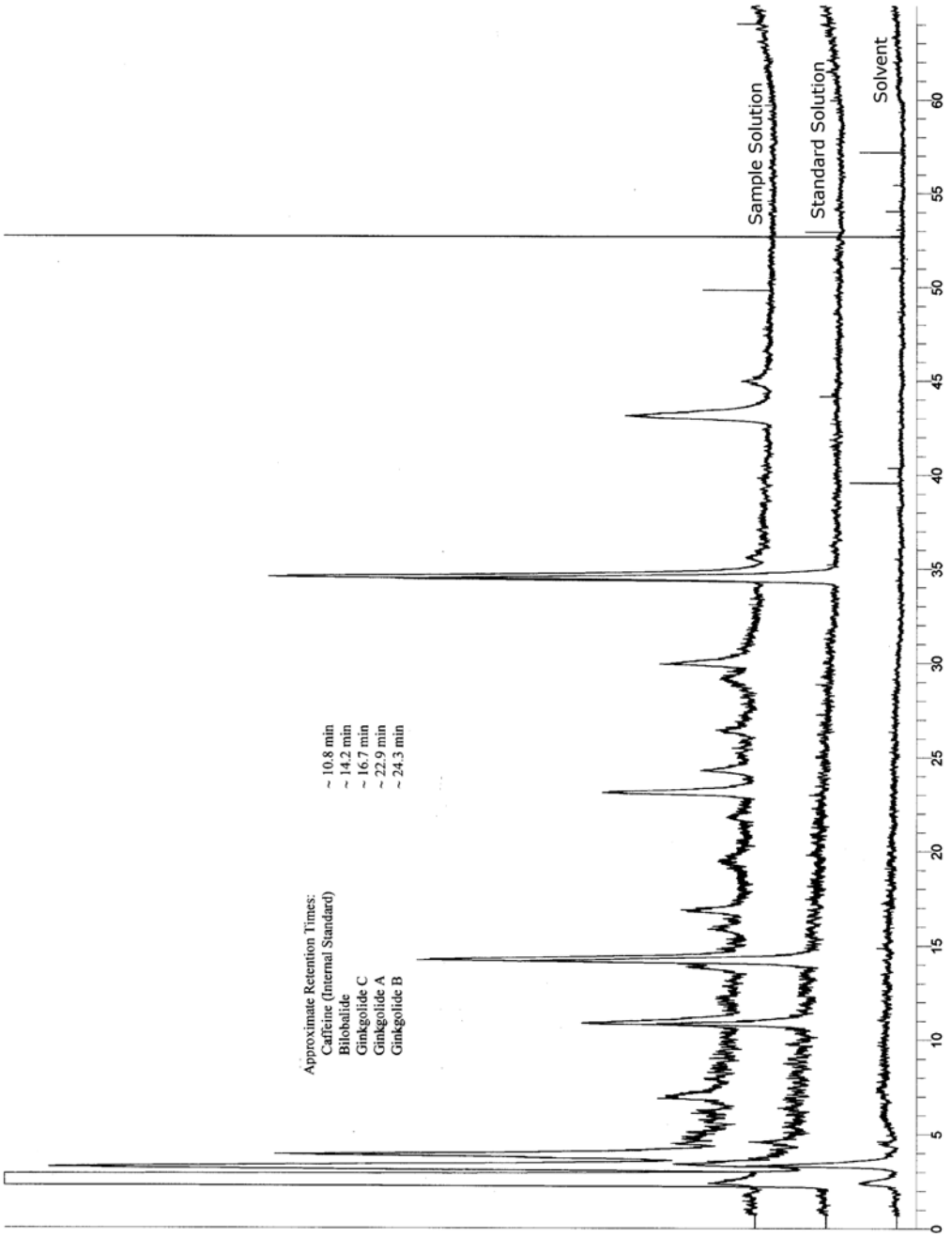

FIGURE I2
High-Performance Liquid Chromatography with Evaporative Light Scattering Detection Quantitation of Terpenoids in *Ginkgo biloba* Extract
Ginkgolides C, A, and B were determined against the bilobalide standard.

TABLE I2
Preparation and Storage of Dose Formulations in the Gavage Studies of *Ginkgo biloba* Extract

3-Month Studies	2-Year Studies
Preparation	
The appropriate amount of test article was weighed into a plastic beaker. Enough corn oil was added to wet the test article, and a smooth slurry was formed by stirring with a spatula. The wetted test article was transferred into a carboy containing corn oil that was being stirred with an overhead stirrer. The plastic beaker, spatula, and sides of the carboy were rinsed with corn oil. The contents of the carboy were diluted to the total final volume with corn oil, and the contents were stirred at a vigorous vortex for approximately 15 minutes with an overhead stirrer. While the formulation was being stirred, approximately 500 mL of it was dispensed into a beaker and poured back into the carboy. The contents of the carboy were stirred at a vigorous vortex for approximately 5 additional minutes with an overhead stirrer. The dose formulations were prepared five times during the studies.	For the 40 and 120 mg/mL dose formulations, the appropriate amount of test article was weighed into a stainless steel mixing bowl. Enough corn oil was added to wet the test article, and the mixture was vigorously stirred with a KitchenAid mixer for approximately 2 minutes. The wetted test article was transferred into a carboy containing corn oil that was being vigorously stirred with an overhead stirrer, and the mixing bowl was rinsed with corn oil until all of the test article was transferred into the carboy. The formulation was vigorously stirred for approximately 5 minutes, and any settled or unwetted test article was scraped with a spatula. While the formulation was being stirred, approximately 200 mL of it were dispensed into a plastic beaker and then poured back into the carboy. The beaker was rinsed with corn oil, and the formulation was diluted to the final volume with additional corn oil, and the mixture was stirred at maximum speed for approximately 5 additional minutes. For the 400 mg/mL formulation, the same procedure was used with the exception that two premixes were prepared in stainless steel mixing bowls and combined into a single carboy. Dose formulations were prepared approximately every 4 weeks.
Chemical Lot Number	
020703	020703
Maximum Storage Time	
35 days	41 days
Storage Conditions	
Stored in sealed plastic bottles enclosed in amber plastic bags at approximately 25° C	Stored in sealed plastic bottles enclosed in amber plastic bags at approximately 25° C
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of *Ginkgo biloba* Extract

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
January 26, 2004	January 30-31, 2004	25	24.01 ± 0.28	–4
		50	50.73 ± 0.81	+1
		100	101.1 ± 2.9	+1
		200	219.9 ± 7.8	+10
		400	411.5 ± 15.0	+3
	March 3-4, 2004[b]	25	25.43 ± 0.38	+2
		50	49.50 ± 1.67	–1
		100	100.0 ± 4.0	0
		200	184.3 ± 8.7	–8
		400	376.6 ± 10.8	–6
	March 8-9, 2004[c]	25	25.07 ± 1.32	0
		50	50.65[d]	+1
		100	94.29 ± 0.46	–6
		200	205.3 ± 8.4	+3
		400	384.3 ± 28.5	–4
February 18, 2004	February 26-27, 2004	25	24.33 ± 0.48	–3
		50	50.64 ± 2.48	+1
		100	105.5 ± 2.5	+6
		200	202.2 ± 12.2	+1
		400	416.6 ± 24.4	+4
	March 29-31, 2004[b]	25	26.01 ± 0.27	+4
		50	49.38 ± 0.83	–1
	April 7-8, 2004[b]	100	104.7 ± 1.8	+5
		200	209.2 ± 11.1	+5
		400	434.3 ± 28.1	+9
	March 29-31, 2004[c]	25	26.23 ± 0.81	+5
		50	50.62 ± 2.85	+1
	April 7-8, 2004[c]	100	103.7 ± 2.5	+4
		200	206.5 ± 4.3	+3
		400	423.8 ± 4.3	+6

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of *Ginkgo biloba* Extract

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
April 12, 2004	April 13-14, 2004	25	26.86 ± 1.33	+7
		50	51.63 ± 3.38	+3
		100	98.62 ± 2.15	–1
		200	201.8 ± 4.0	+1
	April 15-16, 2004	400	417.4 ± 2.7	+4
	May 18-19, 2004[b]	25	27.10 ± 0.61	+8
		50	51.65 ± 1.45	+3
		100	103.2 ± 3.9	+3
		200	202.7 ± 4.2	+1
		400	420.1 ± 24.8	+5
	May 18-19, 2004[c]	25	26.92 ±0.21	+8
		50	52.25[d]	+5
		100	103.5 ± 4.8	+4
		200	212.4 ± 8.91	+6
		400	417.1 ± 15.8	+4

[a] Results of triplicate analyses (mean ± standard deviation). For rats, dosing volume=2.5 mL/kg; 25 mg/mL=62.5 mg/kg, 50 mg/mL=125 mg/kg, 100 mg/mL=250 mg/kg, 200 mg/mL=500 mg/kg, 400 mg/mL=1,000 mg/kg. For mice, dosing volume=5 mL/kg; 25 mg/mL=125 mg/kg, 50 mg/mL=250 mg/kg, 100 mg/mL=500 mg/kg, 200 mg/mL=1,000 mg/kg, 400 mg/mL=2,000 mg/kg.
[b] Animal room samples for rats
[c] Animal room samples for mice
[d] The result from one of the triplicates was a statistical outlier and was excluded.

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of *Ginkgo biloba* Extract

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
February 28, 2005	March 4-5, 2005	40	39.7 ± 0.9	–1
		120	114 ± 1	–5
		400	399 ± 14	0
	April 14-15, 2005[b]	40	40.1 ± 0.6	0
		120	118 ± 5	–2
		400	397 ± 5	–1
May 2, 2005	May 3-4, 2005	40	41.1 ± 0.3	+3
		120	122 ± 0	+2
		400	437 ± 6	+9
July 25-26, 2005	July 28-29, 2005	40	42.0 ± 1.1	+5
		120	125 ± 2	+4
		400	417 ± 4	+4
October 13, 2005	October 24-25, 2005	40	42.9 ± 1.5	+7
		120	130 ± 2	+8
		400	429 ± 7	+7
	November 29-December 1, 2005[b]	40	41.4 ± 1.5	+4
		120	118 ± 7	–2
		400	386 ± 13	–4
January 3, 2006	January 6-7, 2006	40	42.6 ± 0.6	+7
		120	131 ± 2	+9
		400	421 ± 15	+5
March 27, 2006	March 29-30, 2006	40	41.5 ± 0.9	+4
		120	120 ± 3	0
		400	404 ± 12	+1
June 14, 2006	June 16-17, 19-20, 2006	40	43.7± 0.6	+9
		120	124 ± 3	+3
		400	423 ± 4	+6
	July 21-25, 2006[b]	40	38.9 ± 1.1	–3
		120	119 ± 8	–1
		400	388[c]	–3
September 1, 2006	September 6-8, 11, 2006	40	41.2 ± 3.1	+3
		120	127 ± 2	+6
		400	417 ± 18	+4
November 20, 2006	November 22-23, 2006	40	41.7 ± 0.3	+4
		120	124 ± 3	+3
		400	397 ± 7	–1

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of *Ginkgo biloba* Extract

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
February 7, 2007	February 8-9, 2007	40	42.7 ± 1.3	+7
		120	129 ± 0	+8
		400	433 ± 1	+8
	March 16-17, 2006[b]	40	41.1 ± 0.8	+3
		120	128 ± 2	+7
		400	414 ± 6	+4
Mice				
February 28, 2005	March 4-5, 2005	40	39.7 ± 0.9	–1
		120	114 ± 1	–5
		400	399 ± 14	0
	April 14-15, 2005[b]	40	40.9 ± 0.9	+2
		120	119 ± 4	–1
		400	415 ± 7	+4
May 2, 2005	May 3-4, 2005	40	41.1 ± 0.3	+3
		120	122 ± 0	+2
		400	437 ± 26	+9
July 25-26, 2005	July 28-29, 2005	40	42.0 ± 1.1	+5
		120	125 ± 2	+4
		400	417 ± 4	+4
October 13, 2005	October 24-25, 2005	40	42.9 ± 1.5	+7
		120	130 ± 2	+8
		400	429 ± 7	+7
	November 29-December 1, 2005[b]	40	40.5 ± 2.3	+1
		120	128 ± 2	+7
		400	420 ± 31	+5
January 3, 2006	January 6-7, 2006	40	42.6 ± 0.6	+7
		120	131 ± 2	+9
		400	421 ± 15	+5
March 27, 2006	March 29-30, 2006	40	41.5 ± 0.9	+4
		120	120 ± 3	0
		400	404 ± 12	+1
June 14, 2006	June 16-17, 19-20, 2006	40	43.7 ± 0.6	+9
		120	124 ± 3	+3
		400	423 ± 4	+6
	July 21-25, 2006[b]	40	41.5 ± 0.6	+4
		120	118 ± 8	–2
		400	391 ± 13	–2

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of *Ginkgo biloba* Extract

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
September 1, 2006	September 6-8, 11, 2006	40	41.2 ± 3.1	+3
		120	127 ± 2	+6
		400	417 ± 18	+4
November 20, 2006	November 22-23, 2006	40	41.7 ± 0.3	+4
		120	124 ± 3	+3
		400	397 ± 7	–1
February 7, 2007	February 8-9, 2007	40	42.7 ± 1.3	+7
		120	129 ± 0	+8
		400	433 ± 1	+8
	March 16-17, 2006[b]	40	41.6 ± 1.7	+4
		120	123 ± 1	+3
		400	422 ± 10	+6

[a] Results of triplicate analyses. For rats, dosing volume=2.5 mL/kg; 40 mg/mL=100 mg/kg, 120 mg/mL=300 mg/kg, 400 mg/mL=1,000 mg/kg. For mice, dosing volume=5 mL/kg; 40 mg/mL=200 mg/kg, 120 mg/mL=600 mg/kg, 400 mg/mL=2,000 mg/kg.
[b] Animal room samples
[c] The result from one of the triplicates was a statistical outlier and was excluded.

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE J2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 ± 0.68	13.5 – 16.3	25
Crude fat (% by weight)	8.2 ± 0.33	7.6 – 9.3	25
Crude fiber (% by weight)	9.2 ± 0.49	8.4 – 10.0	25
Ash (% by weight)	4.9 ± 0.24	4.6 – 5.4	25
Amino Acids (% of total diet)			
Arginine	0.778 ± 0.068	0.670 – 0.970	21
Cystine	0.220 ± 0.025	0.150 – 0.250	21
Glycine	0.701 ± 0.042	0.620 – 0.800	21
Histidine	0.354 ± 0.079	0.270 – 0.680	21
Isoleucine	0.544 ± 0.045	0.430 – 0.660	21
Leucine	1.092 ± 0.068	0.960 – 1.240	21
Lysine	0.704 ± 0.112	0.310 – 0.840	21
Methionine	0.409 ± 0.047	0.260 – 0.490	21
Phenylalanine	0.626 ± 0.040	0.540 – 0.720	21
Threonine	0.503 ± 0.043	0.430 – 0.610	21
Tryptophan	0.148 ± 0.027	0.110 – 0.200	21
Tyrosine	0.397 ± 0.058	0.280 – 0.540	21
Valine	0.666 ± 0.044	0.550 – 0.730	21
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.227	3.49 – 4.54	21
Linolenic	0.30 ± 0.030	0.21 – 0.35	21
Vitamins			
Vitamin A (IU/kg)	3,737 ± 605	2,340 – 5,080	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	80.1 ± 22.48	27.0 – 124.0	21
Thiamine (ppm)[b]	7.6 ± 0.98	6.3 – 10.5	25
Riboflavin (ppm)	7.1 ± 1.91	4.20 – 11.20	21
Niacin (ppm)	78.6 ± 9.16	66.4 – 98.2	21
Pantothenic acid (ppm)	27.1 ± 12.89	17.4 – 81.0	21
Pyridoxine (ppm)[b]	9.47 ± 2.01	6.4 – 13.7	21
Folic acid (ppm)	1.63 ± 0.49	1.15 – 3.27	21
Biotin (ppm)	0.319 ± 0.10	0.200 – 0.704	21
Vitamin B_{12} (ppb)	53.8 ± 40.6	18.3 – 174.0	21
Choline (ppm)[b]	2,885 ± 459	1,820 – 3,790	21
Minerals			
Calcium (%)	0.960 ± 0.053	0.865 – 1.080	25
Phosphorus (%)	0.554 ± 0.028	0.499 – 0.607	25
Potassium (%)	0.663 ± 0.027	0.626 – 0.732	21
Chloride (%)	0.387 ± 0.039	0.300 – 0.474	21
Sodium (%)	0.190 ± 0.016	0.160 – 0.222	21
Magnesium (%)	0.216 ± 0.063	0.185 – 0.490	21
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	14
Iron (ppm)	185 ± 40.1	135 – 311	21
Manganese (ppm)	51.6 ± 10.49	21.0 – 73.1	21
Zinc (ppm)	53.6 ± 8.62	43.3 – 78.5	21
Copper (ppm)	7.07 ± 2.611	3.21 – 16.30	21
Iodine (ppm)	0.497 ± 0.209	0.158 – 0.972	21
Chromium (ppm)	0.684 ± 0.279	0.330 – 1.380	20
Cobalt (ppm)	0.26 ± 0.164	0.11 – 0.86	19

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.24 ± 0.067	0.16 – 0.40	25
Cadmium (ppm)	0.05 ± 0.007	0.04 – 0.07	25
Lead (ppm)	0.09 ± 0.012	0.07 – 0.12	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.28 ± 0.094	0.18 – 0.49	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	13.44 ± 7.29	4.76 – 36.8	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	10 ± 0.0	10.0 – 10.0	25
Coliform (MPN/g)	3.0 ± 0.0	3.0 – 3.0	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.85 ± 1.85	2.0 – 9.9	25
N-Nitrosodimethylamine (ppb)[e]	2.7 ± 1.22	1.0 – 6.3	25
N-Nitrosopyrrolidine (ppb)[e]	2.2 ± 1.14	1.1 – 6.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.109 ± 0.098	0.020 – 0.356	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.245 ± 0.240	0.020 – 0.997	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

[a] All samples were irradiated. 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] Sources of contamination: alfalfa, grains, and fish meal

[d] Sources of contamination: soy oil and fish meal

[e] 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 sera or feces 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.

In the 3-month studies, serum samples were collected from five male and five female sentinel rats and mice at the midpoint (mice) and end of the study. In the 2-year studies, serum samples were collected from male and female sentinel rats and mice at 6, 12, and 18 months, and from the 1,000 mg/kg groups (rats) and 2,000 mg/kg groups (mice) at the end of the study. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance (Rockville, MD) for determination of antibody titers. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Method and Test	Time of Collection
RATS	
3-Month Study	
ELISA	
PVM (pneumonia virus of mice)	Study start, 1 month, study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	Study start, 1 month, study termination
Sendai	Study start, 1 month, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1 month, study termination
PVM	1 month
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM	Study start, 1, 6, 12, and 18 months, study termination
RCV/SDA	Study start, 1, 6, 12, and 18 months, study termination
Sendai	Study start, 1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1, 6, 12, and 18 months, study termination
PVM	Study start

Method and Test	Time of Collection

MICE
3-Month Study

ELISA	
Ectromelia virus	Study start
EDIM (epizootic diarrhea of infant mice)	Study start
GDVII (mouse encephalomyelitis virus)	Study start
LCM (lymphocytic choriomeningitis virus)	Study start
Mouse adenoma virus-FL	Study start
MHV (mouse hepatitis virus)	Study start
PVM	Study start
Reovirus 3	Study start
Sendai	Study start
Immunofluorescence Assay	
Parvovirus	Study start, 1 month, study termination

2-Year Study

ELISA	
Ectromelia virus	Study start, 1, 6, 12, and 18 months, study termination
EDIM	Study start, 1, 6, 12, and 18 months, study termination
GDVII	Study start, 1, 6, 12, and 18 months, study termination
LCM	Study start, 1, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	Study start, 1 month
Mouse adenoma virus-1	6, 12, and 18 months, study termination
MHV	Study start, 1, 6, 12, and 18 months, study termination
MMV (mouse minute virus, viral protein 2)	Study start, 1, 6, 12, and 18 months, study termination
MPV (mouse parvovirus, viral protein 2)	Study start, 1, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	Study start, 1, 6, 12, and 18 months, study termination
Reovirus 3	Study start, 1, 6, 12, and 18 months, study termination
Sendai	Study start, 1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
MPV, VP2	18 months
EDIM	18 months
MCMV (mouse cytomegalovirus)	Study termination
Polymerase Chain Reaction (PCR)	
Helicobacter species	18 months

RESULTS
All test results were negative.