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U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology Studies of Green Tea Extract in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Green Tea Extract in Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice (Gavage Studies)

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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, 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 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.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

The NTP Technical Reports are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

For questions about the reports and studies, please email NTP or call 984-287-3211.

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This report has been reformatted to meet new NTP publishing requirements;
its content has not changed.

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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 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.

Peer Review

The draft *NTP Technical Report on the on the Toxicology Studies of Green Tea Extract in F344/NTac Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Green Tea Extract in Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice (Gavage Studies)* was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies were appropriate and ensured that this NTP Technical Report presents the experimental results and conclusions fully and clearly.

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Abstract

Dried concentrated extracts from *Camellia sinensis* contain high amounts of catechins and are a popular source for green tea extract nutraceutical supplements and medicinal uses. Supplements containing green tea extract are commonly consumed for weight loss and green tea extracts are popular ingredients in sunblocks, cream rinses, and other cosmetics. Numerous studies in both experimental animals and clinical settings have examined the possible anticancer, anti-inflammatory, anti-microbial, and cardio- and neuroprotective properties of green tea extract. The presumed active ingredient of green tea extract, epigallocatechin gallate (EGCG), was originally nominated by the National Cancer Institute for toxicity and carcinogenicity studies because it is the most abundant catechin in green tea extract, it was being investigated as a potential chemotherapeutic agent, and there was a lack of adequate information with regard to its toxicity. However, NTP selected green tea extract [containing EGCG (48.4% by weight) and other green tea catechins] for study because human exposure is to green tea extract products that contain concentrated mixtures of various green tea catechins. NTP analyzed four lots of green tea extract and selected a source based on quantities of EGCG, consistency with other products on the market, and availability in bulk quantity. Oral gavage was chosen as the route of administration because it was considered most relevant to human exposure. Male and female F344/NTac rats and B6C3F1/N mice were administered green tea extract in water by gavage for 3 months and male and female Wistar Han [Crl:WI(Han)] rats (referred to as Wistar Han rats) and B6C3F1/N mice were administered green tea extract in water by gavage for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes.

Three-month Study in F344/NTac Rats

Groups of 10 male and 10 female core study rats were administered 0, 62.5, 125, 250, 500, or 1,000 mg green tea extract/kg body weight in deionized water by gavage, 5 days per week for 14 weeks. Groups of 10 male and 10 female clinical pathology study rats were administered the same doses for 23 days. One 125 mg/kg female died during week 7. Mean body weights of males and females administered 250 mg/kg or greater were significantly less than those of the vehicle controls.

The cauda epididymis, epididymis, and testes weights of 1,000 mg/kg males were significantly less than those of the vehicle controls. Females administered 1,000 mg/kg had longer estrous cycles and spent significantly more time in extended diestrus than did the vehicle controls. These data indicate that green tea extract exhibited the potential to be a reproductive toxicant in male and female F344/NTac rats.

Several nonneoplastic liver lesions were observed in three of ten 1,000 mg/kg females. Lesions included hepatocyte necrosis, bile duct hyperplasia, oval cell hyperplasia, and mitosis. There were significant increases in the incidences of several nonneoplastic lesions in the nose of 1,000 mg/kg males and females including inflammation (females); hyperplasia in the Bowman's gland of the olfactory epithelium; nerve atrophy; and atrophy, metaplasia, and pigmentation in the olfactory epithelium; the increased incidences of inflammation (females), nerve atrophy, and olfactory epithelium metaplasia and pigmentation (males) were also significant in the 500 mg/kg groups. The incidences of histiocyte cellular infiltration in the mesenteric lymph node in 125 mg/kg or greater males were significantly increased compared to that in the vehicle control group.

Three-month Study in Mice

Groups of 10 male and 10 female mice were administered 0, 62.5, 125, 250, 500, or 1,000 mg green tea extract/kg body weight in deionized water by gavage, 5 days per week for 14 weeks. Six males and four females administered 1,000 mg/kg died before the end of the study; early deaths were due to liver necrosis. Mean body weights of males administered 250 mg/kg or greater and females administered 125 mg/kg or greater were significantly less than those of the vehicle controls. Clinical findings included lethargy, abnormal breathing, and ataxia in females that died early.

A significantly decreased spermatid per testis count was observed in 500 mg/kg males. Females administered 500 mg/kg spent significantly more time in extended diestrus than did the vehicle controls. These data indicate that green tea extract exhibited the potential to be a reproductive toxicant in male and female mice.

In the liver, the incidences of glycogen depletion were significantly increased in 250 and 500 mg/kg males and 500 and 1,000 mg/kg females. In addition, the incidences of centrilobular necrosis were significantly increased in 1,000 mg/kg males and females, and the incidence of karyomegaly was significantly increased in 1,000 mg/kg females. The incidences of nerve atrophy, olfactory epithelium atrophy, and olfactory epithelium metaplasia of the nose were significantly increased in males administered 250 mg/kg or greater and in 500 and 1,000 mg/kg females; the incidence of olfactory epithelium necrosis was significantly increased in 1,000 mg/kg females. The incidences of lymphoid atrophy in the spleen were significantly increased in 500 and 1,000 mg/kg females. The incidences of atrophy of the mandibular lymph node and thymus were significantly increased in 1,000 mg/kg males and females.

Two-year Study in Wistar Han Rats

Groups of 60 male and 60 female rats were administered 0 or 1,000 mg green tea extract/kg body weight and groups of 50 male and 50 female rats were administered 100 or 300 mg/kg in deionized water by gavage, 5 days per week for up to 105 weeks. Ten male and 10 female rats randomly selected from the vehicle control and 1,000 mg/kg groups were evaluated at 3 months; no significant increases in mortality or nonneoplastic lesion incidences were observed at 3 months. In the 2-year study, there were significant decreases in survival in 1,000 mg/kg males and females compared to the vehicle control groups. Mean body weights of 300 and 1,000 mg/kg males were at least 10% less than those of the vehicle control groups after weeks 41 and 9 of the study, respectively; mean body weights of dosed groups of female rats were at least 10% less after weeks 65 (100 mg/kg), 61 (300 mg/kg), and 57 (1,000 mg/kg).

No increases in the incidences of neoplasms in male or female rats were attributed to the administration of green tea extract.

At 2 years, the incidences of hepatic necrosis were significantly increased in 1,000 mg/kg males and females, and the incidence of oval cell hyperplasia was significantly increased in 1,000 mg/kg females.

In the glandular stomach of 1,000 mg/kg males and 300 and 1,000 mg/kg females at 2 years, the incidences of mucosa necrosis were significantly greater than the vehicle control incidences. At 2 years, the incidences of mucosa necrosis in all segments of the small intestine were significantly increased in 1,000 mg/kg males and females.

At 3 months, the incidences of nerve and olfactory epithelium atrophy in the nose in 1,000 mg/kg males and the incidence of pigmentation in the olfactory epithelium of 1,000 mg/kg females were significantly increased. At 2 years, the incidences of numerous nonneoplastic lesions of the nose were generally significantly increased in all dosed groups of males and females. These lesions included mineralization and pigmentation of the lamina propria; suppurative inflammation of the nasopharyngeal duct; nerve atrophy; atrophy, respiratory metaplasia, and pigmentation of the olfactory epithelium; respiratory epithelium atrophy; and deformity and hyperostosis of the turbinate. The incidences of suppurative inflammation were significantly increased in 1,000 mg/kg males and in 300 and 1,000 mg/kg females, and the incidences of basal cell hyperplasia of the olfactory epithelium were significantly increased in males and females administered 300 or 1,000 mg/kg. Incidences of additional nonneoplastic nasal lesions were significantly increased in one or more dosed groups of males and/or females.

The incidences of suppurative inflammation in the lung and inflammation of the heart (epicardium) were significantly increased in 1,000 mg/kg males and females at 2 years.

The incidences of bone marrow hyperplasia in all dosed groups of females were significantly greater than the vehicle control incidence.

The incidences of lymphoid depletion were significantly increased in the spleen of 1,000 mg/kg males and all dosed groups of females.

Two-year Study in Mice

Groups of 50 male and 50 female mice were administered 0, 30, 100, or 300 mg green tea extract/kg body weight in deionized water by gavage, 5 days per week for 105 weeks. Survival of dosed groups was similar to that of the vehicle control groups. Mean body weights of 100 and 300 mg/kg males were at least 10% less than those of the vehicle control group after weeks 89 and 65, respectively, and mean body weights of 100 and 300 mg/kg females were at least 10% less after weeks 25 and 17, respectively.

The incidences of hematopoietic cell proliferation and inflammation in the liver were significantly increased in 300 mg/kg males. The incidences of numerous nonneoplastic lesions of the nose were generally significantly increased in all dosed groups of males and females. These lesions included foreign body, suppurative inflammation, nerve atrophy, atrophy and respiratory metaplasia of the olfactory epithelium, and squamous metaplasia and necrosis of the respiratory epithelium. The incidences of hyperostosis, olfactory epithelium fibrosis, septum perforation, and turbinate atrophy were significantly increased in 100 and 300 mg/kg males and females, and the incidences of respiratory epithelium hyperplasia were significantly increased in 30 and 300 mg/kg males and 100 and 300 mg/kg females. The incidence of nasopharyngeal duct degeneration was significantly increased in 300 mg/kg males.

The incidences of lymphoid hyperplasia and plasma cell infiltration of the mandibular lymph node were significantly increased in 100 and 300 mg/kg males and females.

The incidences of bone marrow hyperplasia were significantly increased in all dosed groups except 30 mg/kg females.

Genetic Toxicology

Green tea extract was mutagenic in *S. typhimurium* strains TA98 and TA100 in the presence of induced rat liver S9; no mutagenicity was observed in these strains without S9 or in the *E. coli* strain WP2 *uvr*A/pKM101, with or without S9. In vivo, no increases in the frequencies of

micronucleated erythrocytes were seen in peripheral blood of male or female B6C3F1/N mice in the 3-month study.

Conclusions

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* (see Explanation of Levels of Evidence of Carcinogenic Activity; see a summary of the peer review panel comments and the public discussion on this Technical Report in Appendix L) of green tea extract in male or female Wistar Han rats administered 100, 300, or 1,000 mg/kg. There was *no evidence of carcinogenic activity* of green tea extract in male or female B6C3F1/N mice administered 30, 100, or 300 mg/kg.

Administration of green tea extract resulted in increased incidences of nonneoplastic lesions of the liver, glandular stomach, small intestine (duodenum, ileum, and jejunum), nose, lung, heart, and spleen in male and female rats; bone marrow of female rats; the nose, mandibular lymph node, and bone marrow of male and female mice; and the liver of male mice.

Synonyms: Green tea catechin polyphenols; green tea; green tea polyphenols

Summary of the Two-year Carcinogenesis and Genetic Toxicology Studies of Green Tea Extract

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Doses in water by gavage	0, 100, 300, or 1,000 mg/kg	0, 100, 300, or 1,000 mg/kg	0, 30, 100, or 300 mg/kg	0, 30, 100, or 300 mg/kg
Survival rates	35/50, 37/50, 43/50, 24/50	26/50, 28/50, 23/50, 4/50	33/50, 36/50, 33/50, 37/50	34/50, 33/50, 44/50, 39/50
Body weights	300 and 1,000 mg/kg groups at least 10% less than vehicle control group after weeks 41 and 9, respectively	100, 300, and 1,000 mg/kg groups at least 10% less than vehicle control group after weeks 65, 61, and 57, respectively	100 and 300 mg/kg groups at least 10% less than vehicle control group after weeks 89 and 65, respectively	100 and 300 mg/kg groups at least 10% less than vehicle control group after weeks 25 and 17, respectively
Nonneoplastic effects	Liver: necrosis (1/50, 2/50, 2/50, 13/50) Stomach, glandular: mucosa necrosis (0/49, 3/50, 3/50, 21/50) Small intestine, duodenum: mucosa, necrosis (0/50, 1/47, 1/49, 10/48) Small intestine, ileum: mucosa necrosis (0/50, 1/48, 2/49, 6/45) Small intestine, jejunum: mucosa necrosis (0/49, 0/47, 2/48, 9/46) Small intestine, (duodenum, ileum, jejunum): necrosis (0/49, 2/46, 4/48, 14/45) Nose: suppurative inflammation (11/50, 12/50, 20/50, 42/50); lamina propria, mineralization (0/50, 33/50, 34/50, 44/50); lamina propria, pigmentation (0/50, 4/50, 11/50, 25/50); nasopharyngeal duct, suppurative inflammation (0/50, 6/50, 8/50, 20/50); nerve, atrophy (0/50, 33/50, 44/50, 44/50); olfactory epithelium, atrophy (1/50, 38/50, 41/50, 41/50);	Liver: necrosis (3/50, 2/48, 5/49, 24/46); oval cell hyperplasia (1/50, 2/48, 3/49, 16/46) Stomach, glandular: mucosa necrosis (0/50, 1/49, 7/49, 20/44) Small intestine, duodenum: mucosa, necrosis (0/47, 0/48, 1/48, 5/39) Small intestine, ileum: mucosa necrosis (0/45, 0/46, 0/47, 5/36) Small intestine, jejunum: mucosa necrosis (0/45, 0/43, 1/45, 6/40) Small intestine, (duodenum, ileum, jejunum): necrosis (0/44, 0/42, 2/44, 10/33) Nose: foreign body (3/49, 2/49, 4/50, 8/49); suppurative inflammation (5/49, 3/49, 17/50, 35/49); epithelium, nasopharyngeal duct, necrosis (0/49, 1/49, 2/50, 7/49); epithelium, nasopharyngeal duct, regeneration (0/49, 0/49, 0/50, 8/49); lamina propria, mineralization (3/49,	Liver: hematopoietic cell proliferation (2/50, 2/50, 6/50, 10/50); inflammation (4/50, 1/50, 5/50, 12/50) Nose: foreign body (1/50, 10/49, 16/50, 25/50); hyperostosis (0/50, 0/49, 28/50, 46/50); suppurative inflammation (14/50, 40/49, 49/50, 48/50); nasopharyngeal duct, degeneration (0/50, 0/49, 4/50, 9/50); nerve, atrophy (0/50, 26/49, 49/50, 50/50); olfactory epithelium, atrophy (4/50, 24/49, 28/50, 3/50); olfactory epithelium, fibrosis (0/50, 4/49, 37/50, 43/50); olfactory epithelium, metaplasia, respiratory (11/50, 45/49, 49/50, 49/50); respiratory epithelium, metaplasia, squamous (0/50, 14/49, 39/50, 46/50); respiratory epithelium, hyperplasia (5/50, 20/49, 10/50, 19/50); respiratory epithelium, necrosis (0/50, 7/49, 16/50, 27/50); septum,	Nose: foreign body (4/48, 8/48, 13/50, 17/50); hyperostosis (0/48, 0/48, 21/50, 48/50); suppurative inflammation (4/48, 24/48, 44/50, 47/50); nerve, atrophy (0/48, 13/48, 47/50, 48/50); olfactory epithelium atrophy (0/48, 18/48, 26/50, 17/50); olfactory epithelium, fibrosis (0/48, 1/48, 39/50, 43/50); olfactory epithelium, metaplasia, respiratory (2/48, 36/48, 49/50, 48/50); respiratory epithelium, hyperplasia (1/48, 1/48, 22/50, 15/50); respiratory epithelium, metaplasia, squamous (0/48, 8/48, 42/50, 42/50); respiratory epithelium, necrosis (0/48, 4/48, 28/50, 32/50); septum, perforation (0/48, 0/48, 38/50, 42/50); turbinate, atrophy (0/48, 0/48, 40/50, 48/50) Mandibular lymph node: lymphoid

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
	olfactory epithelium, hyperplasia, basal cell (0/50, 1/50, 9/50, 28/50); olfactory epithelium, metaplasia, respiratory (4/50, 40/50, 43/50, 47/50); olfactory epithelium, necrosis (1/50, 3/50, 0/50, 12/50); olfactory epithelium, pigmentation (6/50, 18/50, 12/50, 21/50); respiratory epithelium, atrophy (0/50, 2/50, 5/50, 6/50) respiratory epithelium, metaplasia, squamous (0/50, 1/50, 3/50, 7/50); turbinate, deformity (0/50, 16/50, 22/50, 35/50); turbinate, hyperostosis (0/50, 18/50, 27/50, 40/50) Lung: suppurative inflammation (0/50, 1/50, 3/50, 10/50) Heart (epicardium): inflammation (0/50, 0/50, 1/50, 5/50) Spleen: lymphoid depletion (1/50, 2/50, 1/50, 13/50)	23/49, 30/50, 22/49); lamina propria, pigmentation (1/49, 0/49, 6/50, 14/49); nasopharyngeal duct, suppurative inflammation (0/49, 2/49, 5/50, 15/49); nerve, atrophy (0/49, 38/49, 41/50, 38/49); olfactory epithelium, atrophy (2/49, 35/49, 42/50, 34/49); olfactory epithelium, hyperplasia, basal cell (0/49, 0/49, 8/50, 20/49); olfactory epithelium, metaplasia, respiratory (1/49, 42/49, 43/50, 36/49); olfactory epithelium, necrosis (0/49, 3/49, 1/50, 18/49) olfactory epithelium, pigmentation (0/49, 11/49, 7/50, 5/49); respiratory epithelium, atrophy (0/49, 8/49, 9/50, 3/49); respiratory epithelium, necrosis (0/49, 1/49, 2/50, 17/49); respiratory epithelium, pigmentation (0/49, 1/49, 5/50, 5/49); turbinate, deformity (0/49, 6/49, 20/50, 15/49); turbinate, hyperostosis (0/49, 18/49, 32/50, 36/49) Lung: suppurative inflammation (1/50, 3/49, 2/50, 9/48) Heart (epicardium): inflammation (0/50, 2/48, 2/50, 4/48) Bone marrow: hyperplasia (6/50, 14/50, 16/50, 13/50) Spleen: lymphoid depletion (0/50, 7/49, 5/48, 17/43)	perforation (1/50, 0/49, 26/50, 37/50);) turbínate, atrophy (0/50, 0/49, 41/50, 50/50 Mandibular lymph node: lymphoid hyperplasia (0/50, 1/50, 31/50, 37/50); plasma cell infiltration (1/50, 1/50, 24/50, 41/50) Bone marrow: hyperplasia (5/50, 42/50, 38/50, 46/50	hyperplasia (0/50, 1/48, 8/49, 12/48); plasma cell infiltration (0/50, 0/48, 31/49, 18/48) Bone marrow: hyperplasia (6/50, 11/50, 41/50, 34/50)

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Bacterial gene mutations:		Positive in *S. typhimurium* strains TA98 and TA100 with S9, negative in TA98 and TA100 without S9, and negative in *E. coli* with or without S9		
Micronucleated erythrocytes Mouse peripheral blood in vivo:		Negative		

Introduction

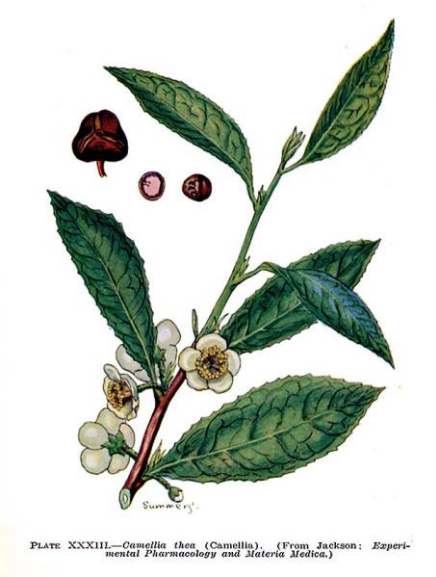

Figure 1. Green Tea (CASRN None Available)

Synonyms: Green tea catechin polyphenols; green tea; green tea polyphenols.
Botanical name: *Camellia sinensis.*

Chemical and Physical Properties

Green tea extract is obtained from leaves of the plant *Camellia sinensis*, of the Theaceae family. *C. sinensis* is indigenous to regions of China, India, and other Southeast Asian countries; however, it is now commercially cultivated in more than 30 countries around the world[1].

The constituents of *C. sinensis* leaves include a complex mixture of polyphenolic compounds (30% to 35%), methylxanthines such as caffeine and theobromine (2.5% to 4.0%), proteins (15% to 20%), amino acids (1% to 4%), carbohydrates (5% to 7%), and lipid components (2%); organic acids (1.5%), ash (5%), minerals and trace elements (10% to 15%); and pigments such as chlorophyll (0.5%)[2]. Among the polyphenolic compounds, flavonoids are the most abundant (80% to 90%). The largest proportion of flavonoids are catechin polyphenols (condensed tannins), which make up 30% to 40% of green tea solids. Catechin polyphenol content depends on the maturity and the processing of the *C. sinensis* leaves during preparation of tea. The catechin polyphenol composition of the leaves also varies with geographic location, season, and cultivation procedures[3]. Catechin polyphenols are colorless, water-soluble compounds and are very stable in acidic solutions (pH less than 4). However, their solubility progressively decreases as the pH is increased from 4 to 8. A typical tea beverage, prepared in a proportion of 1 g leaf/100 mL water in a 3-minute brew, usually contains from 250 to 300 mg tea solids, composed of 30% to 42% catechins and 3% to 6% caffeine[2].

Four major catechins present in green tea extract are epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epicatechin (EC)[2; 3] (Figure 2). EGCG is the most abundant catechin in green tea. Smaller quantities of other catechin polyphenols such as catechin, gallocatechin, epigallocatechin digallate, epicatechindigallate,

3-omethyl EC and EGC, catechin gallate, and gallocatechin gallate are also present in green tea extract.

Production, Use, and Human Exposure

C. sinensis leaves are commonly used for different kinds of tea, such as green tea, oolong tea, and black tea, and the variations in processing these leaves determine the different types of tea produced[3]. Green tea extract used in dietary supplements differs from the common green tea drink in extraction method and the supplements can have a higher concentration of *C. sinensis* components than the traditional tea beverage.

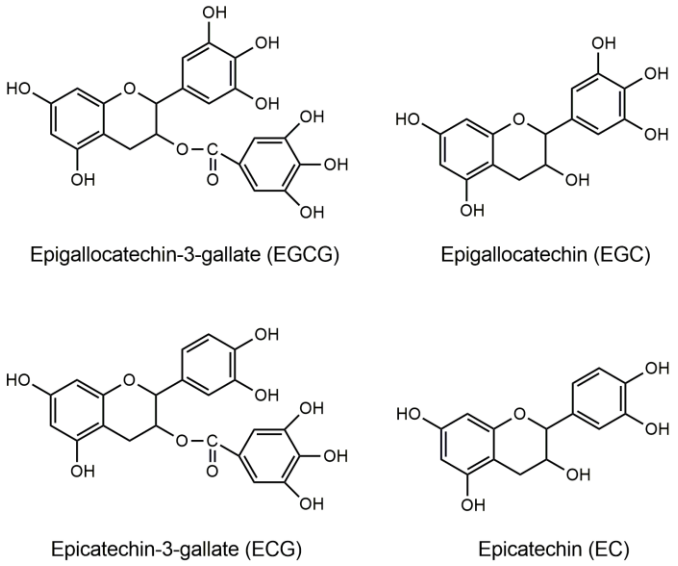

Figure 2. Structures of Major Catechins in Green Tea Extract

Preparation of green tea extract involves steaming of fresh green leaves at high temperatures. This step inactivates the main oxidizing enzyme, polyphenol oxidase, in the *C. sinensis* leaves and preserves the natural chemistry of the catechins during processing[3]. Due to growing commercial interest, the physical and chemical properties of green tea catechins have been extensively studied and, based on the findings, the extraction procedures have been modified and refined. Some of the methods used for extraction of individual catechin polyphenols or a mixture of catechin polyphenols include cold or hot water extraction, organic solvent extraction, microwave-assisted extraction, subcritical water extraction, ultrasound-assisted extraction, and ultra high-pressure extraction[4; 5]. Following extraction, the catechin polyphenols are concentrated to remove extraction solvents. After removal of insoluble material by filtration, the tea infusion with soluble solvents is further concentrated using either a stream of inert gas, a rotary evaporator, a vacuum centrifuge, a vacuum dryer with or without heat, or a freeze dryer to obtain crude green tea extracts in powder form. Although challenging, individual catechin polyphenols (e.g., EGCG, EGC, EC) have also been separated and purified using various methods such as caffeine precipitation, solid-phase extraction, column chromatography, and supercritical fluid extraction[5]. Typically, most green tea extract products are characterized based on the catechin polyphenol content, specifically EGCG, which is thought to be primarily responsible for its pharmacologic activity. In general, catechin polyphenol concentration varies widely among different commercial green tea extract preparations[6; 7].

A United States Pharmacopoeia review lists green tea extract as the fourth most commonly used herbal supplement[7]. Also, an analysis of 2012 United States herbal supplement sales revealed green tea extract to be among the top 20 single and combination dietary supplements[8]. Green tea extracts are consumed as weight loss and dietary supplements and are used as ingredients in sunblocks, cream rinses, and other cosmetics[4; 7]. In 2006, a topical ointment (Veregen™) containing 15% green tea catechin polyphenols was granted market authorization by the FDA for treatment of genital warts. In addition, many studies have investigated potential therapeutic effects of green tea extracts in chemoprevention against various cancers, hypercholesterolemia, atherosclerosis, diabetes, and neurodegenerative and cardiovascular diseases[9-11]. Overall, these studies provide inconsistent evidence on the role of green tea extract in treatment of various diseases and chemoprevention in humans. However, high doses of green tea extract have been shown to be effective in mitigating cancer progression in a few early stage clinical trials. For example, in one clinical trial where 2,000 mg of green tea extract (Polyphenon® E) was administered to cancer patients twice daily for 6 months[12], positive responses were reported in the majority of patients. Other clinical trials examined the effects of green tea extracts on diabetes or cancer with doses ranging from 379 to 2,000 mg administered to participants one or more times daily for 1 to 4 months[13-17]. Nevertheless, very few human studies have been conducted to evaluate long-term safety of green tea extract consumption at high doses.

Regulatory Status

In the United States, dietary supplements such as green tea extract are regulated under the Dietary Supplement Health and Education Act of 1994. Natural extractives, including distillates, of tea are Generally Recognized as Safe Substances[18]. In 2006, the FDA approved a topical ointment (Veregen™) containing 15% green tea catechin polyphenols as the active ingredient for treatment of genital warts in immunocompetent patients 18 years or older[19].

Absorption, Distribution, Metabolism, Excretion, and Toxicokinetics

Experimental Animals

Numerous studies have investigated the absorption, distribution, metabolism, and excretion of green tea extract and individual catechin polyphenols, especially EGCG, in experimental animals. Following gavage administration of 200 mg/kg decaffeinated green tea extract in rats, the peak plasma concentrations (C_{max}) of three major catechin polyphenols (EGCG, EGC, and EC) were reached 1 to 2 hours after administration[20; 21]. The C_{max} of EGCG (16.3 ng/mL) was lower than that of EGC (1,432.8 ng/mL) or EC (685.4 ng/mL); the absolute bioavailabilities were low at 0.1%, 13.7%, and 31.2% for EGCG, ECG, and EC, respectively. When administered alone to rats, EGCG displayed a 3.6-fold higher absorption rate and a higher bioavailability (1.6%) than as a component in green tea extract. Absorption of EGCG was also rapid in mice with a bioavailability of 26% following gavage administration of 75 mg/kg EGCG[22]. In general, the low bioavailability of catechin polyphenols has been attributed to several factors such as catechin instability to digestive conditions[23; 24], transporter-mediated intestinal efflux[25], and rapid metabolism and excretion[26].

Catechin polyphenols were distributed to tissues of rats and mice following gavage or intravenous administration of green tea extract[20; 27]. Higher levels of EGCG, EGC, and EC were reported in the intestine, kidney, and lung[27]. Furthermore, studies in Sprague-Dawley rats have demonstrated that EGCG and other green tea catechins can cross the blood-brain and placental barriers[28-30]. In fasting pregnant Sprague-Dawley rats following gavage administration of 166 mg green tea extract (50% EGCG), maternal plasma concentrations of catechins were about 10 times higher than in the placenta and 50 to 100 times higher than in the fetus[28]. EGCG concentration was higher than the other catechins in maternal plasma and the fetus. Catechin polyphenols were detected in various fetal organs such as brain, eyes, heart, lung, kidney, and liver[31].

The plasma elimination half-lives of catechin polyphenols following gavage administration of green tea extract in rats were 212 minutes, 45 minutes, and 41 minutes for EGCG, EGC, and EC, respectively[21]. When EGCG was administered alone, the half-life was shorter (135 minutes), suggesting that other components in green tea extract could affect the disposition of EGCG. The green tea catechin polyphenols were excreted mainly via feces in rodents with over 80% of the total excreta observed in feces within 24 hours[20; 22]. EGCG is mainly excreted in free form in feces, whereas in urine, greater than 90% of the EGCG was detected in conjugated forms[22].

Similar observations were noted in Sprague Dawley rats administered green tea polyphenols in drinking water; free EGCG was the major catechin found in feces, while EGC and EC were mainly excreted in urine as conjugated forms[27].

In beagle dogs, the elimination half-life of EGCG was 8.6 hours following oral administration of 250 mg/kg of EGCG with an estimated absolute bioavailability of 20%[32]. As observed in rodents, the excretion was predominantly via feces. To mimic chronic consumption, 250 mg/kg of EGCG was administered orally in beagle dogs for 27 days and a 25 mg/kg intravenous dose of [3H]-EGCG was given 1 hour prior to terminal kill[32]. [3H]-EGCG was detected in the tissues including liver (17.47%), small intestine (5.12%), lung (1.16%), and stomach (1.07%). It was also noted that a repeated oral administration resulted in significantly lower blood radioactivity levels compared to a single administration. Repeated administration of green tea extracts to fasted dogs resulted in a two to fourfold increase in the area-under-the-curve (AUC) for catechin polyphenols compared to the fed animals[33].

There is extensive literature on the metabolism of green tea catechin polyphenols both in vitro and in vivo. Phase I metabolism has not been shown to play a major role in the metabolism of green tea catechin polyphenols. However, tea catechin polyphenols undergo significant Phase II metabolism in the liver and intestine to form numerous glucuronidation, sulfation, methylation, and, to some degree, ring fission products[22; 34]. Figure 3 shows the metabolism of the major catechin polyphenol (EGCG) in green tea extract. In rodents and rabbits, other pathways such as glucosidation and thiol conjugation have also been identified[35; 36]. A cysteine conjugate of EGCG has been detected in the urine of rodents following gavage administration of EGCG and was hypothesized to arise as a result of oxidation of EGCG to a quinone metabolite and subsequent reaction with the sulfhydryl group of glutathione. Metabolic degradation of the catechin polyphenols also occurs in the large intestine[36; 37]. In vitro, EGCG-4′-O-glucuronide was identified as the major EGCG metabolite in human, mouse, and rat liver microsomes and homogenates[38-41]. Sulfates of EC and EGCG have been identified in rat liver homogenates[42].

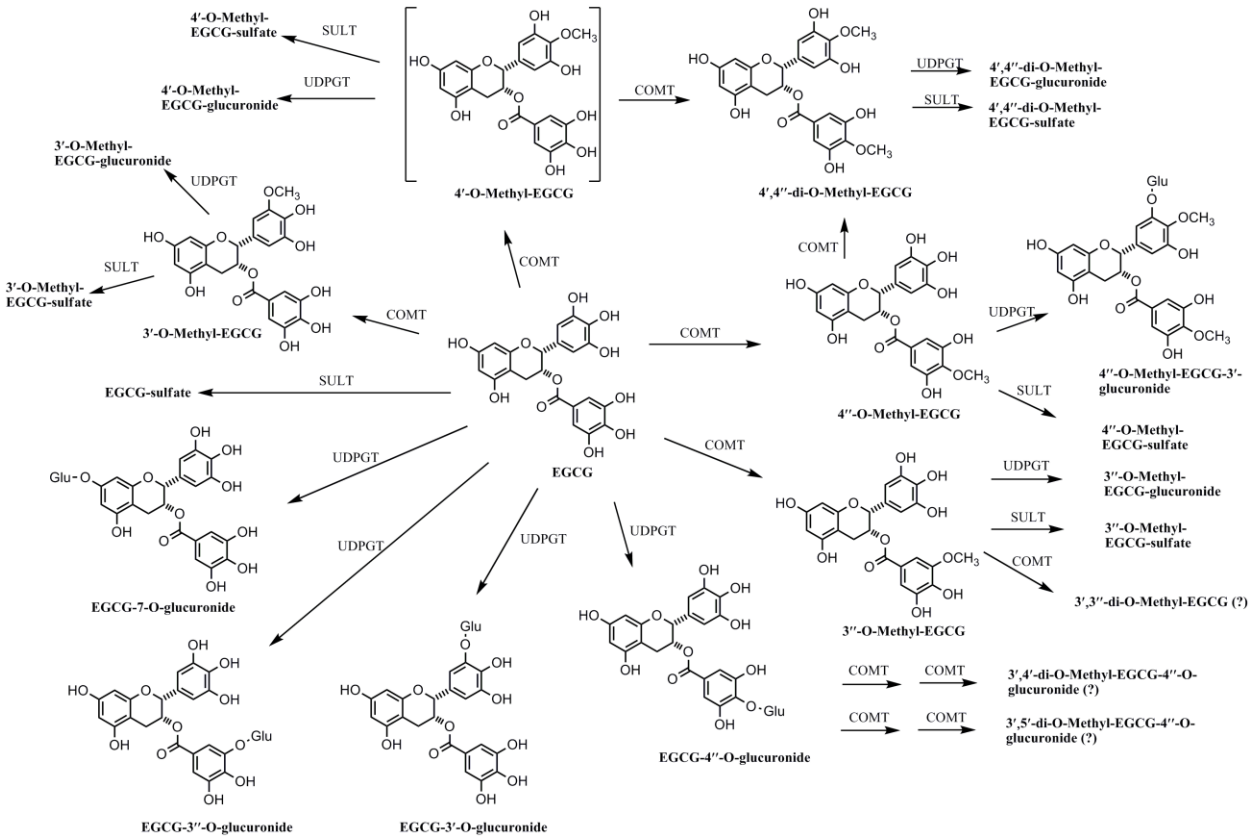

Figure 3. Metabolism of Major Catechins in Green Tea Extract

Adapted from Feng[34].
EGCG = epigallocatechin gallate; COMT = catechol-*O*-methyltransferase; SULT = sulfotransferase; UDPGT = uridine diphosphate glucuronosyltransferase.
Studies on the effect of tea catechin polyphenols on expression of drug transporters and metabolizing enzymes have indicated the inhibition of P-glycoprotein and breast cancer resistance protein activity by EGCG[43-45]. Tea catechin polyphenols have been shown to cause induction of the catalytic activities of CYP1A1, 1A2, 2B, and 3A in both rats and mice, but the results are inconsistent among different studies. Owing to interactions at different stages of metabolism, green tea extract has been reported to affect bioavailability of many drugs such as sunitinib, midazolam, tamoxifen, and verapamil in rats and mice[46-48].

Humans

Pharmacokinetic properties of green tea extract and its catechin polyphenols have been well studied in humans. Orally administered green tea extract and its individual catechins are rapidly absorbed and C_{max} was reached between 1.5 and 2.5 hours in almost all the subjects in various clinical trials[26; 49; 50]. When subjects were administered 664 mg ECG, 459 mg EGC, or 688 mg EGCG, the C_{max} was 3.1, 1.3, or 5.0 μM, respectively[51]. When healthy volunteers were orally administered 50, 100, 200, 400, 800, or 1,600 mg EGCG after overnight fasting, the half-life of elimination ($t_{1/2}$) was between 1.9 and 4.8 hours[50]. Another study investigated the influence of food intake on the absorption of catechin polyphenols in healthy volunteers following ingestion of green tea extract containing up to 1,200 mg EGCG with or without breakfast after overnight fasting[26]. The AUC and C_{max} of EGCG were significantly higher without food than those obtained with food for all three catechins. EGCG was mostly present in the unconjugated form in plasma while EGC and EC were mainly present in the conjugated forms[26]. Catechin polyphenol

metabolism in humans was reported to be similar to that in rodents. Repeated ingestion induced slight accumulation of metabolites. Urinary excretion of catechin polyphenols was low and only 0.1% of ingested EGCG was excreted in urine; more than 90% of the catechins were excreted in feces within 8 hours[52]. Catechin polyphenols have been reported to inhibit catechol-*O*-methyltransferase in human liver microsomes[53]. In vitro studies in human liver microsomes have demonstrated that green tea extract can inhibit CYP2C9, 2D6, and 3A activities[46]. However, the results are mixed and vary depending on the experimental conditions in the different studies. Catechin polyphenols have been reported to inhibit UGT1A1, 1A4, and SULT activities in human liver microsomes[54; 55]. Taken together, these studies suggest that green tea extract may have modulating effects on the pharmacokinetics of coadministered drugs.

Toxicity

Experimental Animals

Acute and subchronic studies on EGCG and green tea extract have been conducted in rats and dogs in accordance with the Organisation for Economic Co-operation and Development (OECD) guidelines[56]. The oral LD_{50} of EGCG (93.4% pure) in Wistar (Crl:WI) BR rats was determined to be between 200 and 2,000 mg/kg and the dermal LD_{50} of EGCG (93%) in HanBrl:WIST (SPF) rats was found to be greater than 1,860 mg/kg.

In a 13-week study, F344/DuCrJ rats were administered green tea extract (Sunphenon 100S™) containing EGCG (29.4%) in feed at 0.3%, 1.25%, or 5.0%[57]. Decreased body weights and significant increases in serum markers of liver toxicity such as AST, ALT, and ALP activities were noted in the 5.0% group. Liver weights were also found to be significantly increased in the 5.0% group; however, there were no corresponding histologic findings. An OECD 408 guideline study was conducted in Sprague-Dawley rats that were administered approximately 50, 150, or 500 mg EGCG preparation (77% EGCG)/kg body weight per day in feed[56] for 13 weeks. There were no treatment-related deaths, signs of systemic toxicity, or organ damage. Lambert et al.[58] treated CF-1 mice under fasting conditions with an intragastric administration of single or multiple once-daily doses of 500 or 750 mg/kg EGCG for 2 to 7 days. There were dose- and time-dependent decreases in survival. The cause of mortality was found to be hepatotoxicity demonstrated by increases in plasma markers of liver damage (ALT) and histopathologic changes in the liver. Male albino rats administered 1.25%, 2.5%, or 5.0% green tea extract (EGCG 9.0%) orally for 30 days showed decreased body weight gains associated with marked hypertrophy and/or hyperplasia of the thyroid gland follicles in the 2.5% and 5.0% groups[59]. Significantly decreased thyroid peroxidase and 5-deiodinase I activities, decreased serum T_3 and T_4 levels, and corresponding increases in TSH levels were observed in the top two dose groups. A 1-year dietary exposure of male and female Wistar Hannover GALAS rats to a catechin mixture (containing 43.6% EGCG) led to an increase in liver weight relative to body weight and centrilobular hypertrophy in the liver at the high dose of 3% in males, but this effect was not observed in females[60].

Studies on Teavigo™ (an EGCG preparation with 90% purity) and Polyphenon® E (containing 56% to 72% EGCG) have demonstrated that the presence of food in the stomach is a major factor that influences toxicity of green tea extract. Oral administration of EGCG (80%) in capsules at doses of 50, 150, or 500 mg/kg per day to beagle dogs under fasting conditions led to vomiting, diarrhea, severe systemic toxicity, and mortality[56]. Histopathologic lesions in the high-

dose groups included necrosis in the liver and kidney, lymphoid atrophy of the thymus, and erosion in the stomach. Total bilirubin was significantly increased in the high-dose groups. Liver necrosis was also observed in one female in the 150 mg/kg group. In the follow-up 13-week study, no evidence of systemic toxicity was observed in dogs administered 50, 300, or 500 mg/kg per day of an EGCG preparation (91% pure) 1 hour after feeding[56]. Similar results have been reported by Kapetanovic et al.[33] following chronic (9 months) administration of 200, 500, or 1,000 mg/kg per day of a purified and standardized green tea extract (Polyphenon® E) (85% to 90% catechins; 56% to 72% EGCG) under fasting conditions. Significant morbidity and mortality were observed. Dose-related increases in weights of the adrenal gland, liver, testis, ovary, spleen, and thyroid/parathyroid glands, and decreases in thymus weights were noted in fasted dogs treated with Polyphenon® E. Macroscopic examination demonstrated grey plaques and mottled and granular lesions in the GI tract and intestine. Histopathologic lesions were observed in multiple organs such as liver, esophagus, stomach, small intestine, kidney, spleen, and lymph nodes. In a follow-up study, 200 mg/kg Polyphenon® E was administered to dogs with and without food for 13 weeks[33]. No mortality was observed in any group. However, animals administered Polyphenon® E on an empty stomach exhibited gastrointestinal irritation as evidenced by vomiting and diarrhea. Histopathologic lesions were observed in the liver, spleen, and bone marrow of the treated dogs.

Humans

A considerable number of case reports and clinical trials provide data on the toxicity of concentrated green tea extract in humans. Many cases of hepatotoxicity have been associated with consumption of high doses of dietary supplements containing green tea extract. In 2003, 13 cases of elevated liver enzymes and hepatotoxicity were associated with consumption of the weight loss supplement Exoliase® in France and Spain[7]. In 12 of these 13 cases the symptoms resolved following discontinuation of use of Exoliase®. However, in one individual (with coadministration of other drugs and alcohol intake), the symptoms did not resolve and the liver damage progressed to liver failure and death. As a consequence, the regulatory authorities of Spain and France suspended marketing authorization for Exoliase®. In a review of the literature, Mazzanti et al.[61] found that 34 cases of hepatitis following consumption of green tea extract were reported from 1998 to 2008. These reports describe cases of 15 patients that consumed green tea extract preparation alone; while the remaining patients had used combination products. Two recent cases of potential green tea extract-induced hepatitis include 81 and 72-year old women who consumed green tea extract (90% EGCG) for 1 month and 3 months, respectively. Clinical manifestation in both cases included vomiting and increases in plasma markers of hepatotoxicity such as ALT, AST, and jaundice. Several adverse event reports have been filed with the FDA describing reactions such as rash, vomiting, diarrhea, tachycardia, cholestatic hepatitis, hepatic failure, increased blood pressure, and myalgia[7].

A few clinical trials associated green tea extract consumption with moderate to severe gastrointestinal and liver toxicity, while some clinical studies reported that green tea extract had no severe adverse events associated with ingestion by humans[13; 62; 63]. Crew et al.[63] conducted a 6-month dose escalation of Polyphenon® E (0, 400, 600, or 800 mg decaffeinated green tea extract containing EGC, EC, ECG, and 65% EGCG) in women suffering from breast cancer to evaluate long-term safety and optimal dose. The authors reported five dose-limiting toxicities, specifically rectal bleeding, weight gain, indigestion, insomnia, and liver function abnormality.

Reproductive and Developmental Toxicity

Experimental Animals

The potential reproductive toxicity of green tea extract has been reported in several studies. Isbrucker et al.[64] conducted an FDA guideline two-generation reproductive toxicity study in male and female Sprague Dawley rats administered 0, 1,200, 3,600, or 12,000 ppm EGCG (91% pure) (target exposures were 0, 100, 300, or 1,000 mg/kg) in the diet for 10 weeks; the animals were then mated. The 12,000 ppm group exhibited an increase in pup loss and lower growth rates in both generations. Exposure-related findings such as thin, unkempt appearance, cyanosis, and weakness were observed in the pups of high dose animals. A slight but significant decrease in thymus weight was observed in the high dose F_1 pups, high dose F_2 male pups, and all exposed groups of F_1 female pups.

Several studies have investigated the developmental toxicity of green tea extract and its active ingredient EGCG in experimental animals. An OECD 414 guideline developmental toxicity study of EGCG was conducted in time-mated pregnant Sprague-Dawley rats[64]. Pregnant dams were administered 1,400, 4,200, or 14,000 ppm [target exposures of 100, 300, or 1,000 mg EGCG (91% pure)/kg body weight per day] in the diet from gestation day 6 through 20. No adverse effects of EGCG administration were apparent on embryo-fetal survival, post-implantation losses, or mean live litter size. No exposure-related external, visceral, or skeletal malformations were noted. A study investigating the developmental toxicity of green tea catechins (30% w/w) administered by gastric intubation to Sprague-Dawley rats demonstrated that there were no fetal malformations or developmental variations related to administration of the test article[65].

Some studies have examined the effects of green tea extracts on cyclophosphamide (CP)-induced teratogenesis. Gavage administration of 100 mg/kg to Sprague-Dawley rats from gestation day 6 through 12 followed by an 11 mg/kg intraperitoneal injection of CP on gestation day 12 resulted in exacerbation of CP-related effects on fetal weights and malformations[66]. Green tea extract alone was not found to increase external, visceral, or skeletal abnormalities in the fetuses. Based on these observations, the authors suggested that upregulation of CYP2B and down-regulation of CYP3A activities by green tea extract led to an enhanced activation and reduced detoxification of CP, thereby exacerbating its teratogenic effects. Pregnant CD-1 mice were administered 100, 200, 400, or 800 mg/kg per day green tea extract alone or with CP (20 mg/kg; intraperitoneal injection on day 10) from gestation day 6 through 13[67]. Given alone, green tea extract was found to increase incidences of microblepharia (abnormal development of eyelids). Administration of 800 mg/kg green tea extract per day with CP to pregnant mice increased the incidences and severities of CP-induced effects such as decreased fetal weights and resorptions. In contrast, moderate doses of green tea extract (200 or 400 mg/kg per day) attenuated the teratogenicity of CP as indicated by decreased incidences of digit, limb, and cranial defects.

Humans

No reports on the reproductive or developmental toxicity of green tea extract in humans were found in the literature.

Immunotoxicity

Experimental Animals

The potential immunotoxicity of green tea extract was investigated by conducting OECD guideline dermal sensitization studies in guinea pigs[56]. Two assays were used; a non-adjuvant method adopted from Beuhler's test and an adjuvant method, based on OECD 406, the guinea pig maximization test (GPMT). During the induction phase of the non-adjuvant assay, 5%, 10%, or 30% green tea extract (EGCG 80%) preparation was applied to the right flank of female GOHI (SPF) guinea pigs 5 days per week. Daily application of the green tea extract showed dose-dependent increases in erythema and irritation during the induction phase suggesting that green tea extract was a dermal irritant. After 4 weeks of daily application, the animals were challenged with 0%, 1%, 3%, 5%, or 10% EGCG on the left flank. A second challenge was performed 2 weeks later at concentrations of 0%, 0.1%, 0.5%, 1%, 3%, 5%, or 10%. The green tea extract preparation induced significantly increased erythema responses in the animals following the second challenge suggesting dermal sensitization. An EGCG preparation (90% EGCG) was also tested in female Himalayan strain albino guinea pigs in a study based on the OECD 406 guideline guinea pig maximization test[56]. On the first day of the induction period, animals were administered intradermal injections of a 1:1 mixture of Freund's complete adjuvant with water, a 0.1% EGCG preparation, or a 1:1 mixture of 0.2% EGCG preparation with Freund's complete adjuvant. On day 8, an area between the injection sites was treated with 0.5 mL of a 50% EGCG solution and kept semi-occluded for 48 hours. The animals were challenged 22 days after the injection of green tea extract by applying 0.15 mL of 50% EGCG solution. The challenge reactions were assessed after 24 and 48 hours. The results demonstrated that 9 of 10 test animals developed a moderate and confluent erythema, indicative of a sensitization reaction. Other studies in mice have demonstrated that the dermal responses to green tea extract could vary depending on the animal strain used and the treatment conditions. Stratton et al.[68] conducted a dermal toxicity study in female BALB/c mice and SKH1 hairless mice topically administered 1%, 3%, or 10% EGCG ointment (w/w) daily for 30 days. Erythema and ulceration were noted in 3% and 10% EGCG treated BALB/c mice that were dehaired using a chemical depilatory agent. However, no toxicity was observed in shaved BALB/c mice exposed to 10% EGCG ointment. Similar to the shaved BALB/c mice, the hairless female SKH1 mice did not show any signs of irritation or dermal toxicity following 30 days of once daily treatment with 10% EGCG ointment. The authors concluded that green tea extract ointment was not an irritant in shaved BALB/c and hairless SKH1 mice except when used with a chemical depilatory agent.

Several studies have also reported immunosuppressive effects of green tea extract in mouse models of autoimmune diseases[69-71]. NOD mice administered 0.2% green tea polyphenols in drinking water for 3 weeks showed significant reductions in serum autoantibody levels, lymphocyte infiltration in submandibular glands, and reduced Sjogren's syndrome-induced cytotoxicity in the salivary glands compared to control mice[71]. Green tea polyphenols (0 or 5 g/L) administered in drinking water for 6 weeks have been reported to decrease ex vivo inflammatory cytokine release from colon explants of treated IL-2−/− mice, an animal model of inflammatory bowel disease[70]. The authors also reported decreases in severities of colitis in treated IL-2−/− mice when evaluated histopathologically. In a collagen-induced mouse model of rheumatoid arthritis, administration of 0.2% green tea polyphenols in drinking water for

12 weeks significantly reduced levels of inflammatory mediators (COX-2, TNF-α, IFN-γ) in the joints and resulted in a greater than 50% reduction of collagen-induced arthritis in the treated mice[69].

Humans

Case reports describing green tea extract-induced asthma in tea factory workers have been published in the literature. These studies also describe immediate dermal and respiratory sensitization responses to powdered green tea extract, crude catechins, EGCG, and noncatechin components[72; 73]. Blood samples from eight workers with green tea-induced asthma showed dose-dependent increases in histamine release following incubation with varying concentrations of EGCG[72].

Carcinogenicity

Experimental Animals

Carcinogenicity of a catechin mixture (containing 43.6% EGCG) was evaluated in Wistar Han GALAS rats[60]. In this study, dietary exposure of the mixture at 0%, 0.2%, 0.3%, 1%, or 3% (0, 8.4, 122.7, 416.4, or 1,265.8 mg/kg per day for male rats and 0, 10.1, 148.4, 476.2, or 1,539.8 mg/kg per day for female rats) was determined to be not carcinogenic at the administered doses. Male F344 rats were coadministered 0.1% or 1.0% green tea extract (EGCG 53.9%) with various carcinogens such as *N*-methyl-nitrosourea or 1,2-dimethylhydrazine for 36 weeks[74]. In the liver, the incidences of tumor nodules were not different between the groups. However, in the pre-neoplastic glutathione-*S*-transferase placental form, positive foci were dose-dependently increased.

Humans

No epidemiological data or case reports describing the carcinogenicity of green tea extract in humans were found in the literature.

Genetic Toxicity

Green tea extracts are complex mixtures of compounds, many of which are bioactive. The composition of the extracts varies depending on the variety and brand of green tea, ratio of tea leaves to water, water quality and temperature, steeping time, whether the brewed tea was lyophilized, and method of extraction from lyophilized tea (e.g., water, ethanol, acetone, ether). Extensive testing of green tea extracts as well as EGCG has produced both positive and negative results in a variety of genotoxicity assays. In addition, green tea extracts have been shown to have antimutagenic effects. With regard to in vivo assays, neither green tea extract nor EGCG were shown to be genotoxic.

In bacterial mutagenicity assays, green tea extract was mutagenic in the absence of exogenous metabolic activation enzymes (S9) in *Salmonella typhimurium* strain TA100 at a lowest effective dose (LED) of 16 mg/plate[75] and in an infrequently used tester strain, *S. typhimurium* BA13 (detects forward mutations via resistance to L-arabinose), in the absence of S9 at a LED of 50 µL/plate[76]. The mutagenicity of green tea extract was not due to caffeine[75; 77]. Conversely, in a number of other bacterial mutation studies, green tea extract was not mutagenic in *S.*

typhimurium TA98, TA100, TA1535, or TA1537 or *Escherichia coli* WP2 *uvrA*[78-80] with or without S9. Additionally, a green tea extract, Polyphenon® E, that is enriched for the catechin (flavanol) group of flavonoids present in green tea leaves was not mutagenic in *S. typhimurium* TA98, TA100, TA1535, or TA1537 or *E. coli* WP2 *uvrA*[81] with or without S9. The highest ineffective doses (HID) of green tea extract or Polyphenon® E tested in these studies were 5,000 μg/plate[80; 81] or 500 mL/plate[79]. In addition to the differences in the concentrations and chemical compositions of various green tea extracts, another complication to assessing the mutagenic potential of green tea extracts arises from the fact that plants can store chemicals as glycones. Some flavonols such as quercetin, kaempferol, and myricetin exist as glycones in tea leaves (Brown and Dietrich[82], reviewed in Spencer[83]), and certain flavonol glycones have been shown to be pro-mutagens in bacterial mutagenicity assays, becoming active only after enzymatic or acidic hydrolysis[78; 82]. Although flavonol glycones appear to resist absorption in the small intestine, their β-glycosidic bonds can be hydrolyzed by mammalian colonic microflora, potentially releasing the mutagenic flavonols[82]. Notably, green tea extract, after being subjected to enzymatic or acid hydrolysis, was shown to be mutagenic in *S. typhimurium* strains TA98 and TA100 in the presence or absence of S9[75; 78]. Therefore, the mutagenicity of green tea extract may be underestimated in bacterial assays due to infrequent use of a catabolism step to release aglycones.

Green tea extract has been tested in several mammalian cell genotoxicity assays. In murine macrophage-like RAW 264.7 cells and human leukemic HL60 cells, green tea extract induced DNA damage as measured by the comet assay (comet tail length) with LEDs ranging from 1 to 4 μg extract/mL in the absence of S9[84]. Micronucleus formation also was induced in the same cell lines as measured by the cytokinesis-block micronucleus assay with LEDs ranging from 12 to 25 μg/mL without S9[84]. Neither study was conducted with S9. Both green tea extract and Polyphenon® E significantly increased the mutant frequency at the *Tk* locus in mouse lymphoma L5178Y cells with or without S9[80; 81]. Chromosomal aberrations were increased after 6 or 24 hours of incubation with green tea extract in Chinese hamster lung cells at a LED of 266.7 μg/mL in the absence, but not the presence, of S9[80].

Green tea extract has been tested for genotoxicity in vivo. Micronucleated polychromatic erythrocytes (MN-PCEs) were not increased in the bone marrow of ICR (Crj: CD-1) mice or Harlan Sprague-Dawley rats exposed to green tea extract (two doses of 2,000 mg/kg delivered by gavage, 24 hours apart)[80] or in bone marrow of ddY male mice following a single exposure to the catechin component of green tea extract (highest ineffective dose of 500 mg/kg by gavage)[85]. In both studies, frequencies of MN-PCEs were measured 24 hours postexposure. Ogura et al.[80] demonstrated that green tea extract was systemically available to mice and rats under nonfasting conditions. Plasma concentrations of total free catechins reached 572 ng/mL or 2,321 ng/mL in male ICR (Crj: CD-1) mice or male Harlan Sprague-Dawley rats (respectively) 30 minutes after a single dose of 2,000 mg/kg green tea extract administered by gavage[80]. In a study in which male and female Swiss Webster mice were given single doses of 0, 375, 750, or 1,500 mg/kg Polyphenon® E by gavage, MN-PCEs from bone marrow were significantly increased ($P < 0.01$) 24 hours after dosing only in the male mice exposed to 375 or 750 mg/kg[81]. Exposure to 1,000 mg/kg per day Polyphenon® E by gavage for 28 days did not increase the frequency of *cII* mutations in the liver, lung, or spleen of male or female B6C3F1 Big Blue Transgenic mice[81].

There has been considerable interest in identifying the specific components of green tea extract that exert genotoxic effects. Research efforts have focused on EGCG, which is considered to be a key active ingredient in green tea extract primarily due to its antioxidant effects, but EGCG has been shown to have pro-oxidant effects, as well[86]. The genotoxicity of a specific EGCG preparation, Teavigo™ (approximately 90% EGCG), has been studied in a number of different assays, and results were mostly negative[87]. Teavigo™ was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA1535, with or without S9, but it was mutagenic at the *Tk* locus in mouse lymphoma L5178Y cells, but only with S9 (LEDs ranged from 125 to 150 μg/mL with different incubation times in Teavigo™). Teavigo™ was negative in the bone marrow micronucleus test in NMRI mice administered a single oral dose (HID was 2,000 mg/kg), in CD-1 mice exposed to Teavigo™ in the diet for 10 days (HID was 12,600 ppm, i.e., 1,200 mg/kg per day), and in Wistar rats given two intravenous injections of Teavigo™ spaced 24 hours apart (HID was 50 mg/kg). Levels of MN-PCEs obtained from bone marrow were assessed 24 hours postexposure for each of these studies. By examining plasma concentrations of Teavigo™, Isbrucker et al.[56] demonstrated that Teavigo™ was orally available to CD-1 mice and that intravenous injection of Teavigo™ into Wistar rats produced plasma levels of EGCG that were 18 to 45 times greater than what could be obtained in humans given an oral dose of 800 mg Teavigo™[50; 88].

The genotoxicity of EGCG has been examined in several in vitro tests and positive results were seen in a number of assays. In murine macrophage-like RAW 264.7 cells and human leukemic HL60 cells, EGCG induced DNA damage in the comet assay (tail length) with LEDs that ranged from 12 to 20 μg/mL[84]. Additionally, EGCG induced DNA damage in the comet assay (tail moment) in freshly isolated human blood lymphocytes at an LED of 1 mM[89; 90]. In the cytokinesis-block micronucleus assay, EGCG induced micronucleus formation in murine macrophage-like RAW 264.7 cells and human leukemic HL60 cells (LEDs ranging from 12 to 25 μg/mL)[84]. However, EGCG did not induce sister chromatid exchanges (SCE) in Chinese hamster ovary K-1 cells (HID of 20 μg/mL)[85] and did not increase ouabain resistance in murine mammary carcinoma FM3A cells at a dose of 50 nmol/mL[91].

Green tea extract and specific chemical components of the extract have been tested far more extensively for their abilities to suppress the genotoxicity of various chemicals than for their genotoxic effects. The majority of this testing has been conducted using bacterial mutagenicity assays although in vitro and in vivo versions of mammalian cell mutation assays, the comet assay, and micronucleus assays have been employed as well. An extensive review of such studies was provided by Gupta et al.[92], and the literature on the antigenotoxic effects of green tea extract has continued to expand considerably since then. Green tea extract and EGCG have shown antigenotoxic effects in the majority of these studies, and several studies have provided mechanistic data (e.g., reduced oxidation of DNA or inhibition of DNA adduct formation) to support these observations[92; 93]. However, many antigenotoxicity studies did not include controls showing the effects of green tea extract or EGCG alone and/or did not include information on cytostatic or cytotoxic effects of the exposures. Therefore, many antigenotoxicity studies are not suitable for drawing definitive conclusions[94].

In summary, green tea extract was positive in some bacterial mutagenicity assays and, along with EGCG, it was positive in a variety of in vitro mammalian cell assays that detect DNA damage, gene mutation, or chromosomal damage. These positive results were obtained mostly in the

absence of S9. However, despite the positive results observed in the in vitro mammalian cell assays, green tea extract and EGCG have not been demonstrated to be genotoxic in any of several in vivo mammalian cell assays for gene mutation or chromosomal damage.

Study Rationale

The purported active ingredient of green tea extract, EGCG, was originally nominated by the National Cancer Institute for toxicity and carcinogenicity studies because it is the most abundant catechin polyphenol in green tea extract, it was being investigated as a potential chemotherapeutic agent, and there was a lack of adequate information with regard to its toxicity. However, NTP selected the green tea extract containing EGCG and other green tea catechin polyphenols as the test article because it was considered more relevant to human exposure and because most commercially available green tea-based products contain a concentrated mixture of various green tea catechin polyphenols. NTP performed 3-month toxicology studies in F344/NTac rats and B6C3F1/N mice and 2-year toxicology and carcinogenicity studies in Wistar Han rats and B6C3F1/N mice using oral gavage administration of green tea extract. Oral gavage was chosen as the route of exposure because it was considered most relevant to human exposure to green tea extract dietary supplements.

Materials and Methods

Procurement and Characterization of Green Tea Extract

Prior to selecting the test article, NTP investigated the composition of four lots of green tea extract from three suppliers. The total catechins determined in the four lots ranged from 62.7% to 83.5% and were in agreement with the total catechin polyphenols reported by the suppliers (72.3% to 83.7%). The epigallocatechin gallate (EGCG) in three of the four lots ranged from 44% to 51%, while in the fourth lot it was 15%. The lot selected for testing was based upon concentration of EGCG, similarity to other products examined by NTP, and availability in bulk quantity.

Green tea extract (ethanol:water extraction of the leaf) was obtained from Amax NutraSource, Inc. (Eugene, OR), in one lot (GTE50-A0302031114) that was used in the 3-month and 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Operations (Columbus, OH) and by the study laboratory at Battelle Columbus Operations (Appendix I). Reports on analyses performed in support of the green tea extract studies are on file at the National Institute of Environmental Health Sciences.

Lot GTE50-A0302031114 of the chemical, a light-brown powder, was identified as green tea extract using infrared spectroscopy. The moisture content of lot GTE50-A0302031114 was determined using weight loss on drying and by Karl Fischer titration. The purity of the test article was determined using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. Components of the purity profile were identified by comparing the retention times from a chromatogram of lot GTE50-A0302031114 to a chromatogram of a solution containing nine potential green tea extract components; these components were quantified by standard addition using authentic standards. HPLC with mass spectrometry (MS) detection was used to confirm the identity of these components.

For lot GTE50-A0302031114, weight loss on drying conducted at two laboratories indicated 4.77% and 5.8% water. Karl Fischer titration conducted at one laboratory indicated 6% to 11.6% water and similar analyses conducted at a second laboratory indicated 3% to 4% water; the latter values were consistent with the 3.32% water indicated in the manufacturer's Certificate of Analysis. HPLC/UV indicated eight components with areas greater than or equal to 1% of the total peak area. Six of these peaks were tentatively identified by matching retention times as epicatechin, catechin gallate, caffeine, epigallocatechin gallate, gallocatechin gallate, and epicatechin gallate. The total area of the major polyphenol peaks represented approximately 84.8% of the total peak area; caffeine constituted 12.27% of the total area, and the remainder was two unidentified components. Nine components of the test article HPLC purity profile, including some below 1%, were identified and quantified by standard addition using the same chromatography system; these components were gallocatechin, epigallocatechin, catechin, epicatechin, catechin gallate, caffeine, epigallocatechin gallate, gallocatechin gallate, and epicatechin gallate. HPLC/MS confirmed the identity of eight of the nine components; epigallocatechin was not identified due to the concentration being below the detection limit in the test article sample. The weight percentage contents of EGCG, epicatechin gallate, epigallocatechin, epicatechin, and caffeine were determined to be 48.4%, 12.8%, 2.26%, 2.83%, and 4.99%, respectively (Table 1); these values were comparable to those listed in the

manufacturer's Certificate of Analysis (53.11%, 13.7%, 2.88%, 3.97%, and 5.42%, respectively). Other catechin polyphenols that were quantified included gallocatechin, catechin, catechin gallate, and gallocatechin gallate, and the weight percentage contents were determined to be 0.52%, 0.51%, 0.45%, and 4.6%, respectively. Taken together, these data indicate that lot GTE50-A0302031114 of the test material was green tea extract.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using HPLC/UV. These studies indicated that green tea extract was stable as a bulk chemical for at least 14 days when stored in sealed amber glass containers at temperatures up to 60°C. To ensure stability, the bulk chemical was stored at room temperature in sealed amber glass containers.

Table 1. Percent by Weight of Various Components Measured in Green Tea Preparations

Class Identified Chemical Components	NIST Standard Reference Material (3255)[a] (%)	NTP Test Article (% gram of extract)[b]	Henning et al. (2003)[c]	Manning and Roberts (2003)[d]	Seeram et al. (2006)[e]	Cabrera et al. (2003)[f]
Catechin polyphenols						
Epigallocatechin gallate (EGCG)	42.2	48.4	28.5	1.08–8.80	3.80–44.4	7.3–10.4
Epicatechin gallate (ECG)	10.0	12.8	7.20	0.48–3.93	1.10–18.6	0.8–2.1
Epigallocatechin (EGC)	8.18	2.26	2.17	1.42–6.80	0.50–9.00	2.4–4.5
Epicatechin (EC)	4.7	2.83	1.97	0.13–1.37	0.80–5.60	0.8–2.1
Gallocatechin (GC)	2.2	0.52	NA	NA	NA	NA
Catechin (C)	0.9	0.51	1.34	0.04–0.50	NA	NA
Gallocatechin gallate (GCG)	3.9	4.6	15.1	0.08–1.09	NA	NA
Methylxanthines						
Caffeine	3.7	4.9	1.63	1.28–4.05	1.10–17.4	2.6–3.8
Theobromine	0.08	NM	NA	NA	NA	NA
Theophylline	0.008	NM	NA	NA	NA	NA
Polyphenol						
Gallic acid	0.32	NM	2.74	NA	NA	0.0–0.2
Amino acid						
Theanine	0.03	NM	NA	NA	NA	NA

NA = not available, NM = not measured.
[a]Sander et al.[95]
[b]Values are from products containing green tea extract alone[6; 96].
[c]Henning et al.[97] evaluated one green tea extract preparation, data originally expressed as mg per g powder.
[d]Manning and Roberts[6] evaluated four green tea encapsulated extracts, data originally expressed as mg per g of capsule.
[e]Seeram et al.[96] evaluated various supplements, dietary supplements with only green tea botanicals provided here (% by tablet weight as reported).
[f]Cabrera et al.[98] evaluated six green tea leaf preparations, data originally expressed as mg per g of tea leaf.

Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 3-month and 2-year studies using HPLC/UV, and no degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing green tea extract with deionized water to give the required concentrations (Table I-2). The dose formulations were stored at room temperature in sealed clear glass bottles, enclosed in amber plastic bags for up to 8 (3-month studies) or 22 (2-year studies) days.

The analytical chemistry laboratory performed a gavageability study of the 200 mg/mL dose formulation using a 25-gauge needle and stability studies of the 6.25 mg/mL dose formulation using HPLC/UV. Gavageability was confirmed and stability was confirmed for at least 42 days for dose formulations stored in sealed amber glass bottles at room temperature and for at least 3 hours under simulated animal room conditions.

The study laboratory performed gavageability studies of the 100 and 200 mg/mL dose formulations using 20- and 18-gauge ball-tipped, stainless steel needles, respectively; in addition, homogeneity studies of the 6.25, 12.5, 100, and 200 mg/mL dose formulations and stability studies of the 6.25, 25, and 200 mg/mL dose formulations were performed using HPLC/UV. Gavageability and homogeneity were confirmed. Stability was confirmed for at least 22 days for dose formulations prepared with sterile water, bottles, caps, and stir bars; formulations were stored at room temperature in clear bottles, but the bottles were placed in amber plastic bags to protect them from light.

Periodic analyses of the dose formulations of green tea extract by measuring EGCG concentrations were conducted by the study laboratory using HPLC/UV. During the 3-month studies, the dose formulations were analyzed three times; all 18 dose formulations were within 10% of the target concentrations (Table I-3). Animal room samples of these dose formulations were also analyzed; all 15 for rats and 14 of 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every two to three months; animal room samples were also analyzed (Table I-4). Of the dose formulations analyzed, all 63 for rats and all 33 for mice were within 10% of the target concentrations; all 12 animal room samples for rats and 12 of 13 for mice were within 10% of the target concentrations.

Animal Source

Male and female F344/NTac rats were obtained from the commercial colony at Taconic Farms, Inc. (Germantown, NY). B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc., for the 3-month studies. Male and female Wistar Han [Crl:WI(Han)] rats were obtained from Charles River Laboratories (Raleigh, NC) and male and female B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc., for use in the 2-year studies. The rationale for change of rat strain from F344/N to F344/NTac was a programmatic decision. For many years NTP used the inbred F344/N rat for its toxicity and carcinogenicity studies. Over a period of time, the F344/N rat exhibited sporadic seizures and idiopathic chylothorax and consistently high rates of mononuclear cell leukemia and testicular neoplasia. Because of these issues in the F344/N rat and NTP's desire to find a more fecund rat

model that could be used in both reproductive and carcinogenesis studies for comparative purposes, a change in the rat model was explored. Following a workshop in 2005, the F344 rat from the Taconic commercial colony (F344/NTac) was used for a few NTP studies to allow NTP time to evaluate different rat models between 2005 and 2006[99]. The Wistar Han rat, an outbred rat stock, was then selected because it was projected to have a long lifespan, resistance to disease, large litter size, and low neonatal mortality.

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. 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 NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Three-month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to green tea extract and to determine the appropriate doses to be used in the 2-year studies.

On receipt, the rats were 3 to 4 weeks old and the mice were 4 to 5 weeks old. Animals were quarantined for 11 or 12 (rats) or 13 or 14 (mice) days; the rats were 5 to 6 weeks old and the 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. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K). All tests results were negative.

Initial doses for the current studies were selected based on a 3-month study in Harlan Sprague-Dawley rats conducted by the National Cancer Institute (NCI). The abstract from that study described increased treatment-related deaths in the high dose group (1,000 mg/kg) administered green tea polyphenols. For the current study, 1,000 mg/kg was chosen as the top dose because it was not known if F344/NTac rats and B6C3F1/N mice were more or less sensitive to green tea extract administration. Also, the green tea extract used in the NCI study was different than that used by NTP for the current study. Hence, there was a possibility of substantial variability in the content of catechin polyphenols in the two extracts and the expected biological response to the two extracts.

Groups of 10 male and 10 female core study rats and mice were administered 0, 62.5, 125, 250, 500, or 1,000 mg green tea extract/kg body weight, in deionized water by gavage 5 days per week for 14 weeks. Vehicle control animals were administered the deionized water vehicle alone; dosing volumes were 5 mL/kg for rats and 10 mL/kg for mice. Groups of 10 male and 10 female clinical pathology study rats were administered the same doses by gavage for 23 days. Feed and water were available ad libitum. Rats and mice were housed individually (male mice) or five per cage. Clinical findings and body weights were recorded 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 the retroorbital plexus of clinical pathology study male and female rats on days 4 and 23 and from the retroorbital plexus (rats) or sinus (mice) of all core study rats and mice at the end of the 3-month studies for hematology and clinical chemistry (rats only) analyses. Animals were anesthetized with a carbon dioxide/oxygen mixture and bled in a random order. Blood was collected into tubes containing EDTA for hematology or into serum separator tubes for clinical chemistry. Hematology parameters were analyzed using the Advia 120 (Bayer Diagnostics Division, Tarrytown, NY). Clinical chemistry parameters were analyzed using the Hitachi 911 (Roche, Indianapolis, IN). 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, 125, 250, and 500 mg/kg groups. The parameters evaluated are listed in Table 2. For 16 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, spleen, 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. In addition, special stains (Schmorl's, PAS, Hall's Bile, and Prussian Blue) were used to determine the type of pigment that occurred in Kupffer cells in the liver of female rats and in histiocytes in the nose of male and female rats. Complete histopathologic examinations were performed by the study laboratory pathologist on all vehicle control and 1,000 mg/kg rats and mice and 500 mg/kg male mice; the heart (mice), liver, lymph nodes, nose, spleen (mice), and thymus were examined to a no-effect level in the remaining groups. Table 2 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) 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 study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[100] and Boorman et al.[101].

Two-year Studies

Study Design

Groups of 60 male and 60 female rats were administered 0 or 1,000 mg green tea extract/kg body weight and groups of 50 male and 50 female rats were administered 100 or 300 mg/kg in deionized water by gavage, 5 days per week for up to 105 weeks. Groups of 50 male and 50 female mice were administered 0, 30, 100, or 300 mg/kg for 105 weeks. Vehicle control animals were administered the sterile water vehicle alone. Ten male and 10 female rats randomly selected from the vehicle control and 1,000 mg/kg groups were evaluated at 3 months to compare the results to the 3-month study in F344/NTac rats. Dosing volumes were 5 mL/kg for rats and 10 mL/kg for mice.

Rats and mice were quarantined for 16 or 17 (rats) or 11 or 12 (mice) 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 were 6 to 7 weeks old and mice 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K). All test results were negative.

Rats were housed three (males) or five (core study and interim evaluation females) per cage and mice were housed individually (males) or five (females) per cage. Feed and water were available ad libitum. 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 of rats and mice were recorded on study day 1, weekly for the first 13 weeks, every 4 weeks thereafter, and at terminal kill. Clinical findings for core study animals were recorded during study week 5, every 4 weeks thereafter, and at study termination.

Complete necropsies and microscopic examinations were performed on all rats and mice. At the 3-month interim evaluation in rats, the heart, right kidney, liver, lung, right testis, and thymus were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin [except, initially, eyes were placed in Davidson's solution and testes (including the epididymides and vaginal tunics) were placed in modified 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. The nasal cavity was sectioned into three levels as described by Boorman et al.[102]. Briefly, Level I is taken at the level of the incisor teeth; Level II is taken midway between the incisors and the first molar; and Level III is taken at the middle of the second molar (between the incisive papilla and the first palatal ridge). Level I is the most rostral

level; Level III the most posterior. 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. During the audit of pathology specimens, the pathologist noted potential lesions in the forestomach, glandular stomach, and small intestine (duodenum, jejunum, and ileum) that had not been previously recorded; therefore a special review was conducted of the wet tissue of the gastrointestinal tract of all rats (including the interim evaluation animals) and mice in the 2-year studies. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the bone, liver, lung, and nose of rats and mice; the heart of rats and male mice; the adrenal cortex, small intestine, kidney, glandular stomach, forestomach, and small intestine of rats; the bone marrow, Harderian gland, and mandibular lymph node of mice; and the pancreas of male 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[100] and Boorman et al.[102]. 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.[103].

In a subsequent, separate evaluation, a complete longitudinal evaluation was done for the residual uterine tissue of rats in the 2-year study. Slides were made of the additional uterine sections and reviewed by a quality assessment pathologist, who also served as the PWG coordinator for these sections.

Table 2. Experimental Design and Materials and Methods in the Gavage Studies of Green Tea Extract

Three-month Studies	Two-year Studies
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species	
F344/NTac rats B6C3F1/N mice	Wistar Han [Crl:WI(Han)] rats B6C3F1/N mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Rats: Charles River Laboratories (Raleigh, NC) Mice: Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days	Rats: 16 (males) or 17 (females) days Mice: 11 (females) or 12 (males) days
Average Age When Studies Began	
Rats: 5 to 6 weeks Mice: 6 to 7 weeks	Rats: 6 to 7 weeks Mice: 5 to 6 weeks
Date of First Dose	
Rats: April 17, 2006 (males) April 18, 2006 (females) Mice: April 19, 2006 (females) April 20, 2006 (males)	Rats: July 18, 2007 (males) July 19, 2007 (females) Mice: July 9, 2007 (females) July 10, 2007 (males)
Duration of Dosing	
5 days/week for 14 weeks	5 days/week for 104 (male rats) or 105 weeks
Date of Last Dose	
Rats: July 17, 2006 (core males) July 18, 2006 (core females) May 9, 2006 (clinical pathology study males) May 10, 2006 (clinical pathology study females) Mice: July 19, 2006 (females) July 20, 2006 (males)	Rats: October 16, 2007 (interim evaluation males) October 17, 2007 (interim evaluation females) July 14, 2009 (core study males) July 16, 2009 (core study females) Mice: July 7, 2009 (females) July 9, 2009 (males)
Necropsy Dates	
Rats: July 18, 2006 (males) July 19, 2006 (females) Mice: July 20, 2006 (females) July 21, 2006 (males)	Rats: October 17, 2007 (interim evaluation males) October 18, 2007 (interim evaluation females) July 13-15, 2009 (core males) July 16-17, 2009 (core females) Mice: July 6-8, 2009 (females) July 8-10, 2009 (males)
Average Age at Necropsy	
Rats: 18 to 19 weeks Mice: 19 to 20 weeks	Rats: 19 to 20 weeks (interim evaluation) 110 to 111 weeks (core study) Mice: 109 to 111 weeks

Three-month Studies	Two-year Studies
Size of Study Groups	
10 males and 10 females	Core study rats and mice: 50 males and 50 females Interim evaluation rats: 10 males and 10 females (0 and 1,000 mg/kg)
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies
Animals per Cage	
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 3 (males) or 5 (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 (male mice) or twice weekly	Same as 3-month studies
Bedding	
Irradiated Sani-Chips® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed once weekly (male mice) or twice weekly	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 sanitized 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: at least 10/hour	Same as 3-month studies

Three-month Studies	Two-year Studies
Doses	
0, 62.5, 125, 250, 500, or 1,000 mg/kg in sterile water [dosing volume 5 mL/kg (rats) or 10 mL/kg (mice)]	Rats: 0, 100, 300, or 1,000 mg/kg in sterile water (dosing volume 5 mL/kg) Mice: 0, 30, 100, or 300 mg/kg in sterile water (dosing volume 10 mL/kg)
Type and Frequency of Observation	
Observed twice daily; core study animals were weighed and clinical findings 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 terminal kill; 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, spleen, right testis, and thymus.	Necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, right testis, and thymus of interim evaluation rats were weighed.
Clinical Pathology	
Blood was collected from the retroorbital plexus of clinical pathology study rats on days 4 and 23 and from the retroorbital plexus (rats) or sinus (mice) of core study rats and mice 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.	None

Three-month Studies	Two-year Studies
Histopathology	
Complete histopathology was performed on all core study vehicle control rats and mice, 1,000 mg/kg rats and mice, and 500 mg/kg male 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), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the heart (mice), liver, lymph nodes, nose, spleen (mice), and thymus were examined to a no-effect level in the remaining groups.	Complete histopathology was performed on all 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), Harderian gland, 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, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.
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, 125, 250, and 500 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 16 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, 125, 250, and 500 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[104] 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[105] method for testing two groups for equality and Tarone's[106] 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 Table A-1, Table A-3, Table B-1, Table B-3, Table C-1, Table C-3, Table D-1, and Table D-3 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 (Table A-2, Table B-2, Table C-2, and Table D-2) 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. Table A-2, Table B-2, Table C-2, and Table D-2 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[107-109] 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[107]. 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[107] following an evaluation of neoplasm onset time distributions for a variety of site- specific neoplasms in control F344/NTac rats and B6C3F1/N mice[110]. Bailer and Portier[107] 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[111].

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). For neoplasms and nonneoplastic lesions detected at the interim evaluation, the Fisher exact test[112], a procedure based on the overall proportion of affected animals, was used.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between 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[113] and Williams[114; 115]. Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[116] (as modified by Williams[117]) and Dunn[118]. Jonckheere's test[119] 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[120] 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[112]. 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[121]. 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 control database must be generally similar. Significant factors affecting the background incidences of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period[122; 123]. In general, the historical database for a given study includes studies using the same route of administration, and the overall incidences of neoplasms in controls for all routes of administration are included for comparison, including the current mouse study. The current study is the only study in Wistar Han rats using water as a gavage vehicle in the historical control

database; therefore, only historical control incidences for all routes and all vehicles are used for Wistar Han rats in this Technical Report.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[124]. In addition, as records from the 3-month and 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assessment 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 green tea 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[125; 126]. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by 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[127] and the somatic mutation theory of cancer[128; 129]. 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[130]. 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)[131; 132]. 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[133; 134]. 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[135]. 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

Three-month Study in F344/NTac Rats

One 125 mg/kg female died during week 7. All other rats survived to the end of the study (Table 3). Final mean body weights and mean body weight gains of males and females administered 250 mg/kg or greater were significantly less than those of the vehicle controls (Table 3 and Figure 4). Animals in the 1,000 mg/kg groups exhibited increased activity for the first 3 weeks of the study.

Table 3. Survival and Body Weights of F344/NTac Rats in the Three-month Gavage Study of Green Tea 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	88 ± 2	340 ± 4	252 ± 4	
62.5	10/10	86 ± 2	330 ± 4	244 ± 4	97
125	10/10	88 ± 2	330 ± 4	242 ± 3	97
250	10/10	87 ± 2	318 ± 4**	231 ± 3**	94
500	10/10	87 ± 2	302 ± 5**	215 ± 5**	89
1,000	10/10	87 ± 2	293 ± 4**	207 ± 4**	86
Female					
0	10/10	81 ± 2	188 ± 2	108 ± 3	
62.5	10/10	81 ± 2	184 ± 3	103 ± 3	98
125	9/10[c]	82 ± 2	184 ± 3	101 ± 3	98
250	10/10	80 ± 2	176 ± 2*	97 ± 2*	93
500	10/10	80 ± 2	179 ± 4*	99 ± 3*	95
1,000	10/10	81 ± 1	176 ± 3**	94 ± 3**	93

*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. Subsequent calculations are based on animals surviving to the end of the study.
[b]Number of animals surviving at 14 weeks/number initially in group.
[c]Week of death: 7.

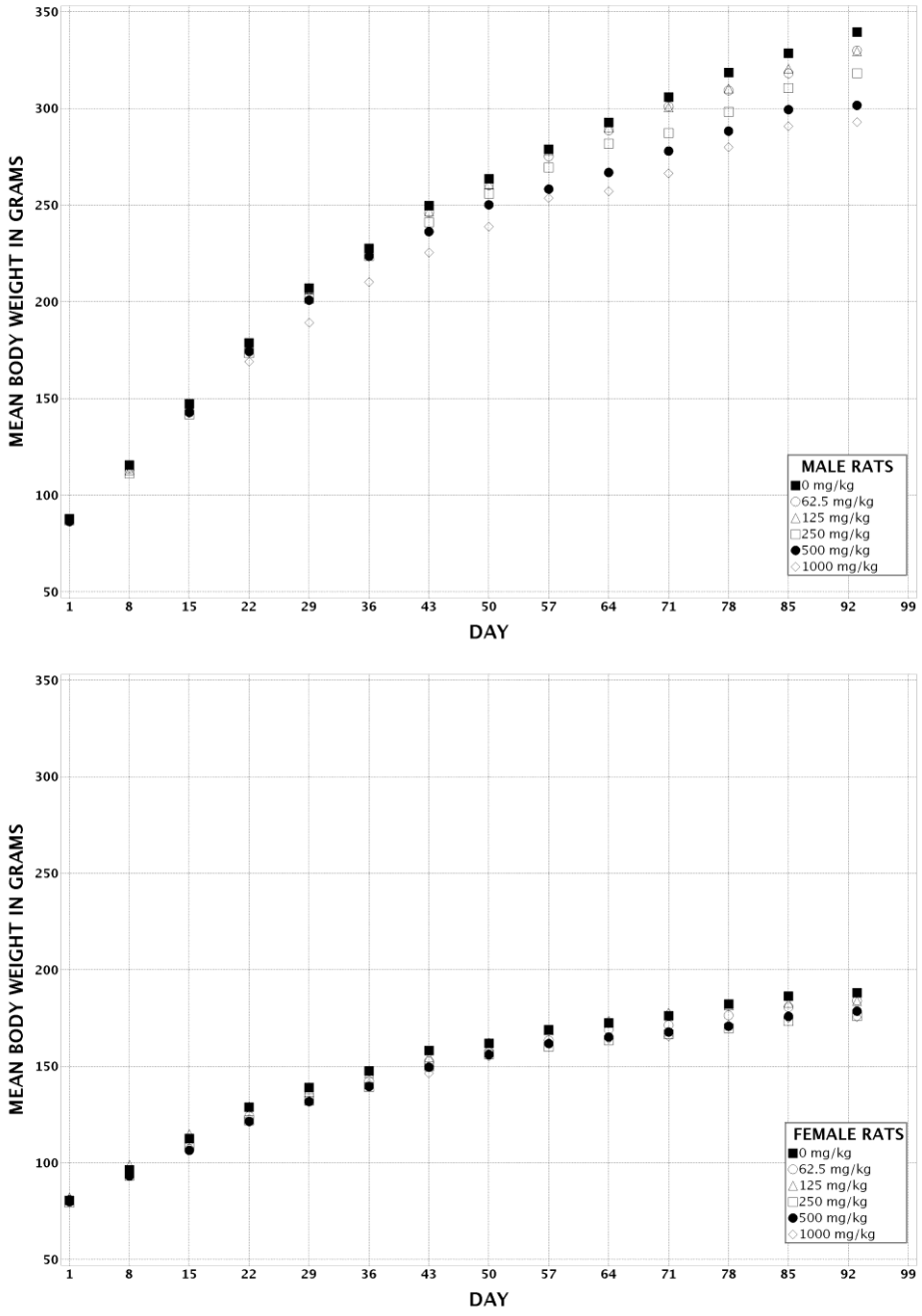

Figure 4. Growth Curves for F344/NTac Rats Administered Green Tea Extract by Gavage for Three Months

The hematology and clinical chemistry data for rats are presented in Table F-1. On day 4, the hematology findings demonstrated a minimal (approximately 5%), transient increase in the circulating erythron (evidenced by increases in hematocrit values, hemoglobin concentrations, and erythrocyte counts) in the 500 mg/kg females and the 1,000 mg/kg males and females. This erythron increase was consistent with a transient, physiologic, hemoconcentration-type response that resolved by day 23.

On days 4 and 23, small increases in alanine amino-transferase activity occurred in the 500 and 1,000 mg/kg males and females; the change resolved by week 14. Because sorbitol dehydrogenase activity, another marker of hepatocellular injury, was not affected at these time points, the transient increases in alanine aminotransferase activity were considered to be related to altered liver metabolism. At week 14, serum concentrations of total bile salts, a marker of hepatic function/injury and/or cholestasis, were increased approximately fivefold in the 1,000 mg/kg groups. Serum alkaline phosphatase activity (another marker of cholestasis), however, was not increased at week 14. Because serum alkaline phosphatase activity was not increased and bile salt concentration can also be affected by hepatic injury or altered function, it appeared the increases in bile salt concentration were not related to a cholestatic event. Interestingly, animal #113, a 1,000 mg/kg female that had an exceptionally high individual bile salt concentration (280 μmol/L) also had an exceptionally high serum alanine aminotransferase activity (7,780 IU/L). Considered together, these two values suggest this animal had treatment-related hepatocellular injury. At week 14, serum total protein and albumin (females only) concentrations were decreased in males and females administered 250 mg/kg or greater.

The absolute lung weights in all dosed groups of males were significantly decreased compared to those of the vehicle control group (up to 20.6% decrease in the 1,000 mg/kg group) (Table G-2). The absolute spleen weights were significantly decreased in all dosed groups of males, with an approximately 25% decrease at 1,000 mg/kg. The relative spleen weight of 1,000 mg/kg males was also significantly decreased (12.7%) when compared to that of the vehicle control group. The absolute testis weight of 1,000 mg/kg males was significantly decreased (6.9%). The absolute thymus weights were significantly decreased in 250 mg/kg and greater males, with a 36.5% decrease in the 1,000 mg/kg group. The relative thymus weight of the 1,000 mg/kg males was significantly decreased (26.2%). In females, the absolute thymus weights were significantly decreased in 500 and 1,000 mg/kg groups, by 17% and 15%, respectively; relative thymus weights were also significantly decreased in 500 and 1,000 mg/kg females, by 12% and 9.5%, respectively. There was an increase in the incidence of seminiferous tubule degeneration of the testes in the 1,000 mg/kg males when compared to the vehicle control group, although the increase was not significant by a pairwise comparison. There were significant increases in the incidences of atrophy of the thymus in 1,000 mg/kg males and females when compared to the vehicle control groups. Other tissues with organ weight changes had no correlating histopathologic changes. Although the absolute liver weight decreased in males, the decreases paralleled decreases in body weights. Despite histologic changes in the livers of three 1,000 mg/kg females, the liver weights were similar to vehicle controls.

Males administered 1,000 mg/kg exhibited significantly lower cauda epididymis (12%), epididymis (10%), and testis weights (4%) compared to those of the vehicle controls (Table H-1). Total sperm per cauda exhibited a negative trend (14% lower in the 1,000 mg/kg group). Females administered 1,000 mg/kg displayed an increase in estrous cycle length and spent significantly more time in extended diestrus than did the vehicle controls (Table H-2). Thus, under the conditions of these studies, green tea extract administered by oral gavage exhibited the potential to be a reproductive toxicant in male and female F344/NTac rats.

Relevant findings in the liver were limited to three female rats in the 1,000 mg/kg group (Table 4). Lesions included a single case of moderate hepatocyte necrosis characterized by necrotic hepatocytes, hemorrhage, and a mononuclear infiltrate in the centrilobular regions. The nuclei of necrotic hepatocytes frequently had pyknotic and karyorrhectic nuclei and either

vacuolated or eosinophilic condensed cytoplasm. The necrosis was associated with mild chronic inflammation. Additional lesions in female rats included minimal bile duct hyperplasia, minimal to mild oval cell hyperplasia, and minimal to mild mitosis reflecting an increased number of hepatocytes with mitotic figures located in the midzonal regions between the periportal and centrilobular areas, as well as mild accumulation of golden brown pigment in Kupffer cells (consistent with ceroid lipofuscin and glycoprotein with special staining). Two female rats, including the one female rat with necrosis, were diagnosed with periportal hypertrophy. The hypertrophic hepatocytes also had karyomegaly.

Table 4. Incidences of Nonneoplastic Lesions of the Liver in Female F344/NTac Rats in the Three-month Gavage Study of Green Tea Extract

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Number Examined Microscopically	10	10	10	10	10	10
Chronic Inflammation[a]	0	0	0	0	0	1 (2.0)[b]
Mitoses	0	0	0	0	0	2 (1.5)
Bile Duct, Hyperplasia	0	0	0	0	0	3 (1.0)
Hepatocyte, Necrosis	0	0	0	0	0	1 (3.0)
Oval Cell, Hyperplasia	0	0	0	0	0	3 (1.7)
Periportal, Hypertrophy	0	0	0	0	0	2 (1.5)

[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Significantly increased incidences of lesions in the nose were limited to males and females in the 500 and 1,000 mg/kg groups and included inflammation (females); hyperplasia of the glands underlying the olfactory epithelium (Bowman's glands); nerve atrophy; and atrophy, metaplasia, and pigmentation of the olfactory epithelium (Table 5). Other biologically important lesions, which were not significantly increased, included occasional occurrences of inflammation (males); pigmentation within histiocytes of the lamina propria; degeneration of the nasopharyngeal duct epithelium and inflammation within the nasopharyngeal duct lumen (males); hyperplasia of the basal cells of the olfactory epithelium; olfactory epithelium necrosis (males); and atrophy, squamous metaplasia, hyperplasia, and necrosis of the respiratory epithelium (primarily in males). These isolated lesions most likely represent different manifestations of the same pathologic process within the nose, and they are considered part of the spectrum of changes within the nasal cavity that was related to exposure to green tea extract.

Grading criteria for the nasal lesions were as follows: minimal was characterized by unilateral involvement, mild represented bilateral involvement or unilateral on multiple levels, moderate represented bilateral involvement on more than one level; severe represented involvement of all or almost all of the respective epithelium (e.g., olfactory) present in all levels.

Increases in incidence and/or severity of inflammation were noted in males and females in the 500 and 1,000 mg/kg groups and in the 250 mg/kg females. This change was characterized by largely neutrophilic infiltrates, with lesser numbers of lymphocytes and plasma cells in the lamina propria, migrating through the epithelium and sometimes filling the airways or extending

into Bowman's glands. Inflammation frequently involved the respiratory epithelium of the nasal septum or lateral wall of Levels I and II and the olfactory epithelium in the dorsal or dorsolateral meatus of Levels II and III. In severe instances only among the males treated with 500 or 1,000 mg/kg, the infiltrate was associated with olfactory epithelium necrosis**,** characterized by sloughing or loss of the olfactory epithelium with pyknosis and karyorrhexis of the nuclei.

Nasopharyngeal duct degeneration was noted at Level III in 500 and 1,000 mg/kg males and in 500 mg/kg females and was often accompanied by inflammation in males. Nasopharyngeal duct degeneration was characterized by decreased goblet cells and transformation of tall columnar ciliated epithelial cells to a more attenuated or cuboidal cell. If present, the inflammatory infiltrate was largely neutrophils with lesser numbers of mononuclear cells.

Hyperplasia of Bowman's glands located in the olfactory epithelium was diagnosed in 500 and 1,000 mg/kg males and females and in 250 mg/kg females, and the incidences in 1,000 mg/kg males and females were significantly greater than those in the vehicle control groups. Bowman's gland hyperplasia was characterized by nodular proliferation of cells that occasionally formed an acinus in the lamina propria underlying or continuous with the olfactory epithelium. The finding was mostly in the dorsal meatus or upper portion of the septum in Level II and occasionally in Level III.

Olfactory epithelial atrophy was noted in the 62.5, 125, and 1,000 mg/kg females and in males in the 125 mg/kg or greater groups. Olfactory epithelial atrophy was associated with thinning of the layers of olfactory epithelial cells, especially in the dorsal meatus, compared to vehicle controls. Nerve atrophy was noted in males and females administered 500 or 1,000 mg/kg and in one female administered 250 mg/kg. This change was characterized by the loss of olfactory nerve bundles in the submucosal regions underlying the olfactory epithelium in the dorsal meatus of Level II and dorsal meatus and nasal turbinates of Level III. Nerve atrophy was frequently accompanied by olfactory epithelium metaplasia characterized by the transition of olfactory epithelium to respiratory epithelium. Metaplasia of the olfactory epithelium was present in males in the 125, 500, and 1,000 mg/kg groups and in females in the 500 and 1,000 mg/kg groups.

Table 5. Incidences of Nonneoplastic Lesions of the Nose in F344/NTac Rats in the Three-month Gavage Study of Green Tea Extract

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
Number Examined Microscopically	10	10	10	10	10	10
Inflammation[a]	2 (1.0)[b]	3 (1.0)	1 (1.0)	2 (1.0)	3 (1.3)	5 (2.0)
Glands, Olfactory Epithelium, Hyperplasia	0	0	0	0	3 (1.3)	7** (1.1)
Lamina Propria, Pigmentation, Histiocyte	0	0	0	0	0	2 (2.0)
Nasopharyngeal Duct, Degeneration	0	0	0	0	3 (2.0)	3 (2.0)
Nasopharyngeal Duct, Inflammation	0	0	0	0	2 (1.0)	3 (2.0)
Nerve, Atrophy	0	0	0	0	5* (1.8)	10** (1.7)
Olfactory Epithelium, Atrophy	0	0	2 (1.0)	1 (1.0)	3 (1.0)	9** (1.1)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	0	0	0	1 (1.0)	1 (1.0)
Olfactory Epithelium, Metaplasia	0	0	1 (1.0)	0	6** (1.5)	10** (1.0)
Olfactory Epithelium, Necrosis	0	0	0	0	1 (1.0)	3 (1.7)
Olfactory Epithelium, Pigmentation	0	0	0	0	4* (1.0)	5* (1.0)
Respiratory Epithelium, Atrophy	0	0	0	0	0	1 (2.0)
Respiratory Epithelium, Hyperplasia	1 (2.0)	0	0	0	2 (1.0)	4 (1.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	0	1 (1.0)
Respiratory Epithelium, Necrosis	0	0	0	0	0	1 (2.0)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Inflammation	2 (1.0)	1 (1.0)	1 (1.0)	4 (1.0)	10** (1.2)	8* (1.0)
Glands, Olfactory Epithelium, Hyperplasia	0	0	0	2 (1.0)	1 (1.0)	4* (1.0)
Lamina Propria, Pigmentation, Histiocyte	0	0	0	0	0	1 (1.0)
Nasopharyngeal Duct, Degeneration	0	0	0	0	2 (1.5)	0
Nerve, Atrophy	0	0	0	1 (1.0)	4* (1.0)	5* (1.8)
Olfactory Epithelium, Atrophy	0	1 (1.0)	1 (1.0)	0	0	7** (1.1)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	0	0	1 (1.0)	0	0

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Olfactory Epithelium, Metaplasia	0	0	0	0	5* (1.2)	4* (1.0)
Olfactory Epithelium, Pigmentation	0	0	0	2 (1.0)	3 (1.0)	5* (1.0)
Respiratory Epithelium, Hyperplasia	0	0	0	1 (1.0)	1 (1.0)	0

*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 lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Basal cell hyperplasia of the olfactory epithelium was diagnosed in one 500 and one 1,000 mg/kg male and one 250 mg/kg female. Basal cell hyperplasia was characterized by a minimal proliferation (two to three cells thick) of basal cells at the margin of the lamina propria and the olfactory epithelium in the dorsal meatus.

Increased incidences, when compared to that in the vehicle controls, of respiratory epithelium hyperplasia were diagnosed in the 500 and 1,000 mg/kg males, and single incidences were seen in females administered 250 or 500 mg/kg. This lesion was characterized by the proliferation of nonciliated cuboidal epithelium more than three cell layers thick in Level I or occasionally Level II involving the nasal septum or lateral wall.

Atrophy and necrosis of the respiratory epithelium were diagnosed in one male in the 1,000 mg/kg group. The presence of golden brown pigment accumulation in the degenerating olfactory epithelium was noted in 500 and 1,000 mg/kg males and in females administered 250 mg/kg or greater, in addition to the sporadic cases of golden brown pigmented histiocytes in the lamina propria in 1,000 mg/kg males and females. Pigmented cells in the nose stained positive for Schmorl's and PAS but negative for Prussian Blue and Hall's, suggesting that the pigment was composed of a combination of glycoprotein and lipofuscin.

Squamous metaplasia of the respiratory epithelium was noted in one 1,000 mg/kg male and was characterized by transformation of single-layer tall columnar epithelial cells to well-differentiated keratinized epithelium four to nine layers thick unilaterally in Level II along the ventral portion of the nasal septum.

Atrophy of the mesenteric lymph node was seen in one 1,000 mg/kg female and was characterized by loss of lymphocytes and numerous lymphocytes undergoing apoptosis in the paracortex. The incidences of histiocyte cellular infiltration in the mesenteric lymph node in 125 mg/kg or greater males were significantly increased compared to that in the vehicle control group [vehicle control, 0/10; 62.5 mg/kg, 2/10 (2.0); 125 mg/kg, 6/10 (1.5); 250 mg/kg, 7/10 (1.9); 500 mg/kg, 7/10 (1.7); 1,000 mg/kg, 7/10 (1.7)].

Minimal thymus atrophy was noted in males and females administered 1,000 mg/kg [males: 0/10, 0/10, 0/10, 1/10 (1.0), 0/10, 5/8 (1.0); females: 0/10, 0/10, 0/10, 0/10, 0/10, 6/10 (1.0)]; one 250 mg/kg male also had this lesion. Thymus atrophy was characterized by variable thinning of the cortex due to loss of T cells by apoptosis. Thymus atrophy was considered related to stress.

Degeneration of the seminiferous tubules of the testes was increased in 1,000 mg/kg males compared to the vehicle control males, although this increase was not significant by a pairwise comparison, only with a trend test [3/10 (1.0), 1/10 (1.0), 2/10 (1.0), 3/10 (1.0), 3/10 (1.0), 7/10 (1.0)]. Seminiferous tubule degeneration was characterized by vacuolated or absent germinal epithelial cells, resulting in decreased layers of germinal epithelium. In a few cases, tubules were completely devoid of germinal epithelium, and had only Sertoli cells remaining.

Dose Selection Rationale: All rats survived until the end of the 3-month study. Treatment-related body weight effects and histopathologic lesions of minimal to mild severity were not considered dose limiting. Therefore, green tea extract doses selected for the 2-year gavage study in Wistar Han rats were 100, 300, and 1,000 mg/kg.

Two-year Study in Wistar Han Rats

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 5). The survival of 1,000 mg/kg males and females was significantly less than that of the vehicle controls. Early mortality may have been related to gastrointestinal lesions, respiratory compromise, or cardiovascular collapse.

Table 6. Survival of Wistar Han Rats in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Male				
Animals initially in study	60	50	50	60
3-month interim evaluation[a]	10	–	–	10
Accidental deaths[a]	0	0	0	2
Moribund	12	7	5	5
Natural deaths	3	6	2	19
Animals surviving to study termination	35	37	43[b]	24
Percent probability of survival at end of study[c]	70	74	86	50
Mean survival (days)[d]	687	695	705	591
Survival analysis[e]	P = 0.001	P = 0.755N	P = 0.085N	P = 0.037
Female				
Animals initially in study	60	50	50	60
3-month interim evaluation[a]	10	–	–	10
Accidental deaths[a]	0	0	1	3
Moribund	14	12	17	8
Natural deaths	10	10	9	35
Animals surviving to study termination	26	28	23	4
Percent probability of survival at end of study	52	56	47	9
Mean survival (days)	671	646	566	408
Survival analysis	P < 0.001	P = 0.910N	P = 0.394	P < 0.001

[a]Censored from survival analyses.
[b]Includes one animal that died during the last week of the study.
[c]Kaplan-Meier determinations.
[d]Mean of all deaths (uncensored, censored, and terminal kill); does not include interim evaluation animals.
[e]The result of the life table trend test[106] is in the vehicle control column, and the results of the life table pairwise comparisons[105] with the vehicle controls are in the dosed group columns. A lower mortality in a dose group is indicated by **N.**

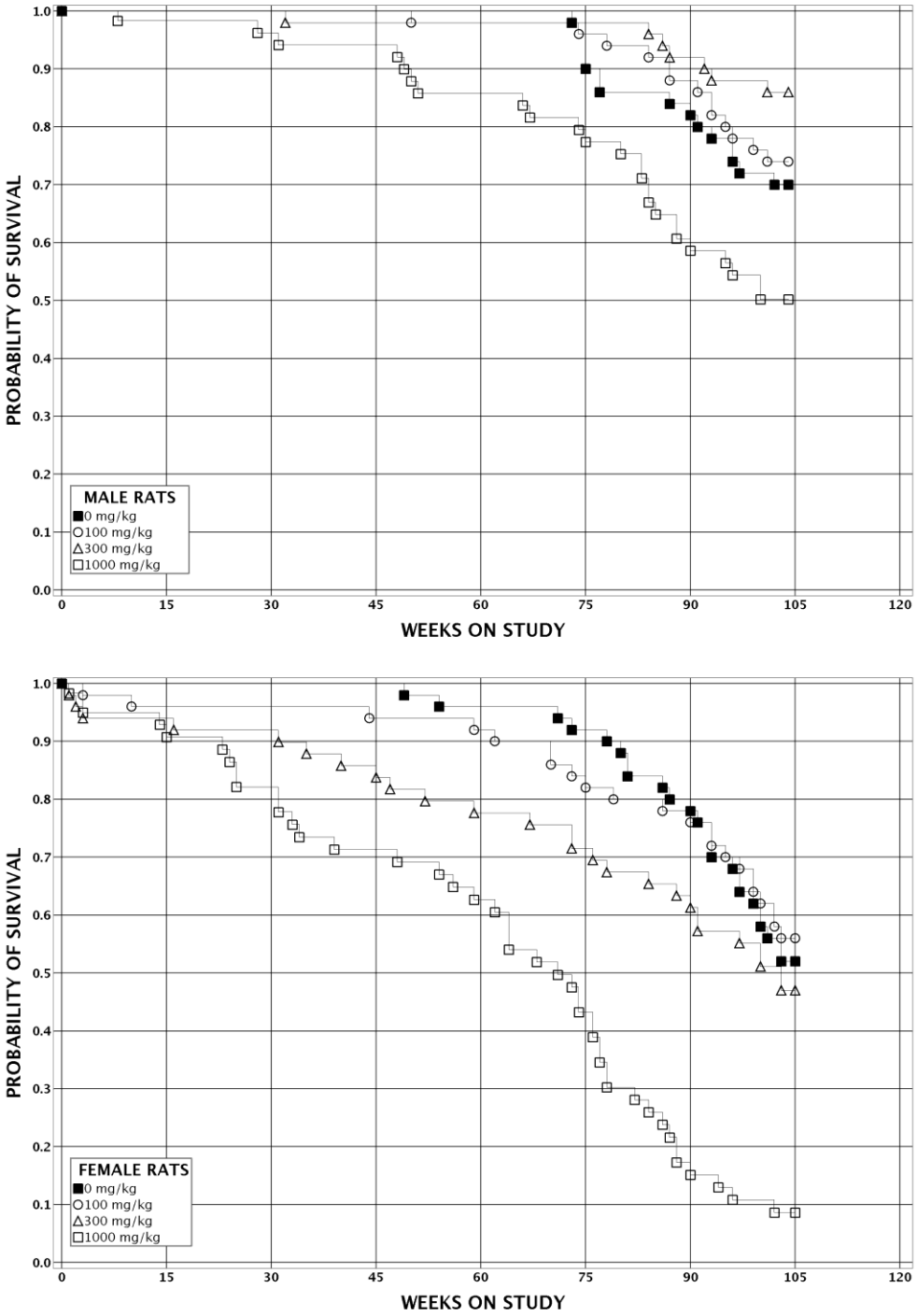

Figure 5. Kaplan-Meier Survival Curves for Wistar Han Rats Administered Green Tea Extract by Gavage for Two Years

Body Weights, Organ Weights, and Clinical Findings

Mean body weights of 300 and 1,000 mg/kg males were at least 10% less than those of the vehicle control group after weeks 41 and 9 of the study, respectively; mean body weights of dosed groups of female rats were at least 10% less after weeks 65 (100 mg/kg), 61 (300 mg/kg), and 57 (1,000 mg/kg) (Table 7, Table 8, and Figure 6). At the 3-month interim evaluation, body weights in the vehicle control and 1,000 mg/kg groups were similar for both males and females. The relative kidney weight of 1,000 mg/kg males was significantly greater than that of the vehicle control group (Table G-2); this change was not observed in the 3-month study in F344/NTac rats, and there were no histopathologic findings that correlated to this change. The absolute and relative thymus weights of 1,000 mg/kg females were significantly less than those of the vehicle controls (approximately 16%), but this decrease was less than that seen in the 1,000 mg/kg females in the 3-month study in F344/NTac rats. In the interim evaluation animals, there were no histopathologic changes in the thymus to correlate to the decrease in thymus weights, in contrast to the thymic atrophy observed in the 3-month study. No clinical findings related to chemical exposure were observed.

Table 7. Mean Body Weights and Survival of Male Wistar Han Rats in the Two-year Gavage Study of Green Tea Extract

Day	0 mg/kg		100 mg/kg			300 mg/kg			1,000 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	167	60	169	101	50	167	100	50	167	100	60
8	210	60	209	99	50	207	99	50	204	97	60
15	251	60	250	99	50	245	98	50	237	95	60
22	282	60	281	100	50	272	96	50	263	93	60
29	306	60	306	100	50	296	97	50	283	93	60
36	327	60	326	100	50	315	96	50	301	92	60
43	342	60	341	100	50	330	97	50	314	92	60
50	358	60	356	100	50	342	96	50	328	92	60
57	368	60	367	100	50	352	96	50	335	91	59
64	381	60	379	99	50	362	95	50	341	89	59
71	390	60	388	100	50	370	95	50	349	90	59
78	399	60	395	99	50	377	95	50	355	89	59
85	407	60	402	99	50	381	94	50	360	89	59
113	435	50[a]	422	97	50	397	91	50	373	86	47[a]
141	460	50	445	97	50	421	92	50	394	86	47
169	475	50	458	97	50	433	91	50	397	84	47
197	493	50	470	95	50	445	90	50	416	84	46
225	504	50	484	96	50	458	91	49	422	84	45
253	516	50	493	96	50	467	91	49	430	83	45
281	529	50	508	96	50	479	91	49	437	83	45
309	542	50	519	96	50	485	90	49	441	82	45
337	557	50	530	95	50	491	88	49	446	80	44
365	569	50	545	96	49	499	88	49	457	80	41
393	577	50	557	97	49	508	88	49	465	81	41
421	589	50	561	95	49	508	86	49	471	80	41
449	598	50	569	95	49	514	86	49	470	79	41
477	605	50	577	95	49	526	87	49	478	79	39
505	611	49	583	96	49	534	88	49	482	79	39
533	618	45	582	94	48	534	87	49	486	79	37
561	620	43	587	95	47	542	87	49	485	78	36
589	627	43	591	94	46	547	87	48	483	77	32
617	632	42	601	95	44	548	87	46	487	77	29
645	625	39	597	96	42	546	87	45	477	76	28
673	633	37	593	94	39	550	87	44	482	76	26
701	632	36	591	93	38	561	89	43	480	76	24
Mean for Weeks											
1–13	322	–	321	100	–	309	96	–	295	92	–
14–52	501	–	481	96	–	453	90	–	417	83	–
53–101	610	–	580	95	–	532	87	–	477	78	–

[a]Interim evaluation occurred during week 14.

Table 8. Mean Body Weights and Survival of Female Wistar Han Rats in the Two-year Gavage Study of Green Tea Extract

Day	0 mg/kg		100 mg/kg			300 mg/kg			1,000 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	129	60	129	99	50	129	100	50	130	100	60
8	148	60	148	100	50	149	101	49	148	100	59
15	166	60	166	100	49	166	100	48	165	99	57
22	179	60	179	100	49	177	99	47	176	99	56
29	189	60	189	100	49	186	98	47	189	100	56
36	198	60	199	100	49	194	98	47	196	99	56
43	204	60	206	101	49	202	99	47	205	101	56
50	210	60	212	101	49	208	99	47	211	101	56
57	214	60	216	101	49	213	100	47	214	100	56
64	219	60	220	101	48	217	99	47	220	101	56
71	222	60	224	101	48	220	99	47	223	100	56
78	225	60	223	99	48	218	97	47	226	100	56
85	227	60	226	99	48	222	98	47	227	100	55
113	235	50[a]	234	100	48	230	98	45	231	98	42[a]
141	242	50	242	100	48	240	99	45	243	100	42
169	246	50	243	99	48	242	99	45	245	100	40
197	252	50	248	98	48	249	99	45	252	100	38
225	258	50	251	97	48	250	97	44	254	99	36
253	263	50	255	97	48	252	96	43	258	98	34
281	269	50	260	97	48	260	97	42	261	97	33
309	276	50	264	96	47	264	96	41	266	97	33
337	285	50	270	95	47	269	94	40	267	94	32
365	292	49	273	94	47	271	93	39	271	93	32
393	298	48	279	94	47	277	93	39	276	93	30
421	308	48	283	92	46	281	91	38	276	90	29
449	318	48	290	91	45	287	90	38	278	88	25
477	326	48	293	90	45	288	88	37	286	88	24
505	339	47	297	87	43	288	85	37	285	84	23
533	345	46	299	87	41	289	84	34	296	86	17
561	354	43	309	87	40	301	85	33	304	86	14
589	360	42	312	87	40	309	86	32	292	81	12
617	365	40	313	86	39	311	85	31	282	77	8
645	366	36	313	86	37	307	84	28	285	78	7
673	367	34	320	87	35	313	85	28	277	76	5
701	370	28	321	87	31	318	86	25	275	74	5
Mean for Weeks											
1–13	195	–	195	100	–	192	98	–	195	–	100
14–52	258	–	252	98	–	251	97	–	253	–	98
53–101	339	–	300	88	–	295	87	–	283	–	83

[a]Interim evaluation occurred during week 14.

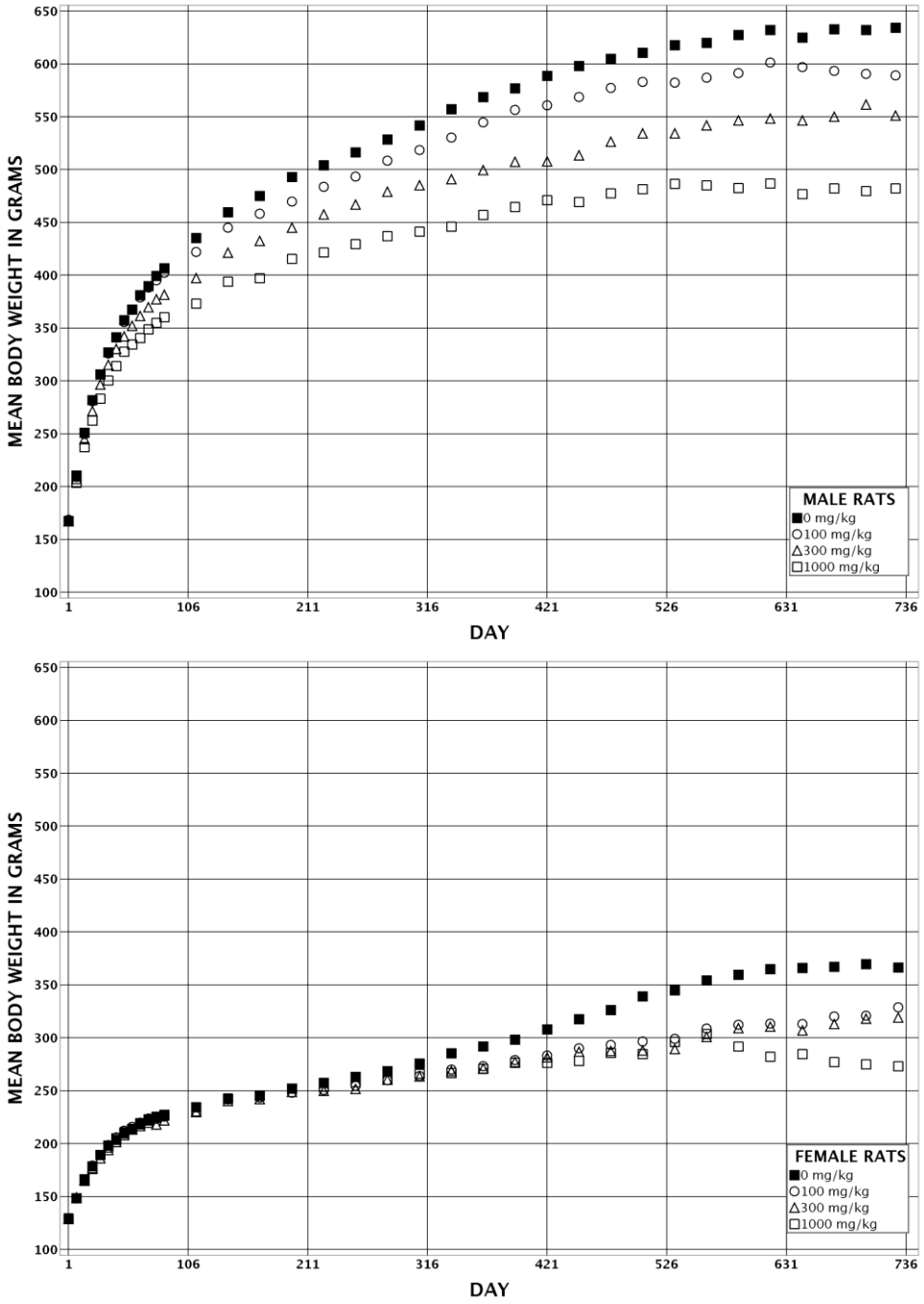

Figure 6. Growth Curves for Wistar Han Rats Administered Green Tea Extract by Gavage for Two Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms or nonneoplastic lesions of the liver, stomach, small intestine (duodenum, ileum, and jejunum), nose, lung, heart, bone marrow, spleen, pituitary gland, mammary gland, and uterus. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats.

Liver: At the 3-month interim evaluation, one 1,000 mg/kg female had several nonneoplastic changes in the liver, mostly of minimal severity, that were not seen in the vehicle control females (Table 9 and Table B-3).

Table 9. Incidences of Nonneoplastic Lesions of the Liver in Wistar Han Rats in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Male				
Two-year Study				
Number Examined Microscopically	50	50	50	50
Necrosis[a]	1 (1.0)[b]	2 (1.0)	2 (1.5)	13** (2.9)
Oval Cell Hyperplasia	0	0	0	2 (1.5)
Female				
Three-month Interim Evaluation				
Number Examined Microscopically	10	–	–	10
Inflammation	0	–	–	3 (1.0)
Necrosis	0	–	–	1 (2.0)
Bile Duct Hyperplasia	0	–	–	1 (1.0)
Hepatocyte Hypertrophy	0	–	–	1 (1.0)
Oval Cell Hyperplasia	0	–	–	1 (1.0)
Two-year study				
Number Examined Microscopically	50	48	49	46
Necrosis	3 (2.7)	2 (2.0)	5 (2.6)	24** (3.3)
Oval Cell Hyperplasia	1 (1.0)	2 (1.0)	3 (1.7)	16** (1.3)

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

These included inflammation, necrosis, bile duct hyperplasia, hepatocyte hypertrophy, and oval cell hyperplasia. Two other animals from the same group had only inflammation. Necrosis was characterized by individual scattered hepatocytes, hypereosinophilia, variable cell body swelling or shrinking, and variable nuclear karyorrhexis/karyolysis. Hypertrophy was characterized by enlargement of the hepatocytes due to an increase of finely granular eosinophilic cytoplasm.

Inflammation was composed of mixed inflammatory cell infiltrates including lymphocytes, plasma cells, and neutrophils both in portal regions as well as multifocally scattered throughout the hepatic parenchyma. Oval cell hyperplasia was composed of multifocal proliferations of small cells with scant cytoplasm and ovoid nuclei occasionally forming clusters and linear tracts. The formation of distinct ducts was characteristic of bile duct hyperplasia. The lesions were similar to those observed in three female F344/NTac rats in the 3-month study. There were no differences in liver weights of dosed groups compared to those of the vehicle controls.

At 2 years, the incidences of necrosis and oval cell hyperplasia were generally significantly increased in 1,000 mg/kg males and females (Table 9, Table A-3, and Table B-3).

The liver necrosis was characterized by both necrosis of individual hepatocytes and submassive to massive necrosis of hepatic parenchyma. There was no distinct pattern to the necrosis, though the less severe lesions were predominantly midzonal. Individual cell necrosis was characterized by rounding up and shrinkage of individual hepatocytes with nuclear condensation-pyknosis. In more severe lesions, individual cell necrosis was characterized by swollen, hypereosinophilic hepatocytes with nuclear karyolysis-karyorrhexis; these lesions were associated with congestion, hemorrhage, predominantly neutrophilic cellular infiltrates, and hypertrophy/hyper-plasia of the Kupffer cells. These lesions in the 300 and 1,000 mg/kg males and all dosed groups of females could be distinguished from the liver necrosis diagnosed in vehicle control males and females and 100 mg/kg males by the presence of large numbers of individual necrotic hepatocytes and/or the presence of congestion/hemorrhage. Acute hepatic necrosis may have been related to septic shock or hypoxia rather than direct hepatotoxicity. Oval cell hyperplasia was primarily minimal and was characterized by increased cellularity, primarily within portal areas, with proliferation of small cells with scant cytoplasm and oval-shaped nuclei occasionally forming linear tracts, though not forming ducts.

Gastrointestinal Tract: At 2 years, dose-related increased incidences of gray to black, focal to diffuse discoloration of the mucosa and wall of the stomach and/or variable lengths of the small intestine were observed macroscopically (Figure 10).

In the glandular stomach of 1,000 mg/kg males and 300 and 1,000 mg/kg females, the incidences of mucosa necrosis were significantly greater than the vehicle control incidences (Table 10, Table A-3, and Table B-3). Mucosa necrosis was characterized by a variably thick zone of mucosa with shrunken, hypereosinophilic cytoplasm and nuclear pyknosis, which was accompanied by cell debris, congestion, hemorrhage, fibrin thrombi, and bacterial colonies in older lesions. There were small numbers of treated animals with increased numbers of pigment-laden cells, and occasionally the pigment was darker brown and globular, consistent with hemosiderin and suggestive of prior hemorrhage.

Dose-related increased incidences of mucosa necrosis were seen in all the segments of the small intestine, and the incidences in 1,000 mg/kg males and females were significantly greater than those in the vehicle control groups (Table 10, Table A-3, and Table B-3). The severity of this lesion generally increased with increasing dose. Mucosa necrosis was characterized by findings that varied from excessive exfoliation of shrunken, hypereosinophilic epithelial cells with pyknotic nuclei at the tips (or lateral aspect) of villi or variable lengths of shriveled intact villous epithelium lacking supporting lamina propria (Figure 11). In addition to changes to the mucosal epithelium, the capillaries of the subjacent lamina propria of affected villi were often markedly

dilated and infrequently contained fibrin thrombi. Occasionally remaining villi showed evidence of regeneration characterized by unevenly spaced epithelial cells with variable amounts of cytoplasm and vesicular nuclei. One female in the 100 mg/kg group had hemorrhagic necrosis of the small intestine mucosa. This lesion had a different appearance than mucosa necrosis, in that it was hemorrhagic and involved the entire thickness of the intestinal wall, rather than just the mucosa. Similar lesions were not observed in the interim evaluation animals.

Table 10. Incidences of Mucosa Necrosis of the Gastrointestinal Tract in Wistar Han Rats in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Two-year Study				
Male				
Stomach, Glandular[a]	49	50	50	50
Mucosa, Necrosis[b]	0	3 (2.0)[c]	3 (1.7)	21** (2.5)
Intestine Small, Duodenum	50	47	49	48
Mucosa, Necrosis	0	1 (1.0)	1 (2.0)	10** (2.4)
Intestine Small, Ileum	50	48	49	45
Mucosa, Necrosis	0	1 (1.0)	2 (1.5)	6** (1.7)
Intestine Small, Jejunum	49	47	48	46
Mucosa, Necrosis	0	0	2 (1.0)	9** (2.0)
Intestine Small (Duodenum, Ileum, or Jejunum)	49	46	48	45
Mucosa, Necrosis	0	2	4	14**
Two-year Study				
Female				
Stomach, Glandular	50	49	49	44
Mucosa, Necrosis	0	1 (2.0)	7** (1.1)	20** (1.9)
Intestine Small, Duodenum	47	48	48	39
Mucosa, Necrosis	0	0	1 (1.0)	5** (2.2)
Intestine Small, Ileum	45	46	47	36
Mucosa, Necrosis	0	0	0	5** (1.0)
Intestine Small, Jejunum	45	43	45	40
Mucosa, Necrosis	0	0	1 (1.0)	6** (1.0)
Intestine Small (Duodenum, Ileum, or Jejunum)	44	42	44	33
Mucosa, Necrosis	0	0	2	10**

**Significantly different ($P \leq 0.01$) from the vehicle control group by the Poly-3 test.
[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; no average severity grades available for combined small intestine sites.

Nose: In 1,000 mg/kg males and females at 3 months, lesions included suppurative and acute inflammation; nerve atrophy; and olfactory epithelium atrophy, respiratory metaplasia, and pigmentation (Table 11, Table A-3, and Table B-3). Other lesions that occurred only in 1,000 mg/kg males were hyperplasia and necrosis of the nasopharyngeal duct epithelium and suppurative inflammation in the nasopharyngeal duct; mineralization in the lamina propria; olfactory epithelium necrosis; and squamous metaplasia of the respiratory epithelium. In 1,000 mg/kg females, additional lesions were degeneration and regeneration of the epithelium of the nasopharyngeal duct, acute inflammation in the nasopharyngeal duct, pigmentation in the lamina propria, and turbinate deformity.

Table 11. Incidences of Nonneoplastic Lesions of the Nose in Wistar Han Rats in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Male				
Three-month interim evaluation				
Number examined microscopically	10	–	–	10
Inflammation, suppurative[a]	0	–	–	2 (3.0)[b]
Inflammation, acute	0	–	–	1 (1.0)
Epithelium, nasopharyngeal duct, hyperplasia	0	–	–	1 (3.0)
Epithelium, nasopharyngeal duct, necrosis	0	–	–	1 (2.0)
Lamina propria, mineralization	0	–	–	1 (1.0)
Nasopharyngeal duct, inflammation, suppurative	0	–	–	2 (3.5)
Nerve, atrophy	0	–	–	5* (2.0)
Olfactory epithelium, atrophy	0	–	–	5* (2.0)
Olfactory epithelium, metaplasia, respiratory	0	–	–	3 (1.7)
Olfactory epithelium, necrosis	0	–	–	2 (1.0)
Olfactory epithelium, pigmentation	0	–	–	3 (1.3)
Respiratory epithelium, metaplasia, squamous	0	–	–	2 (1.0)
Two-year study				
Number examined microscopically	50	50	50	50
Inflammation, suppurative	11 (1.7)	12 (1.8)	20 (2.0)	42** (2.2)
Epithelium, nasopharyngeal duct, degeneration	0	1 (2.0)	0	3 (2.7)
Epithelium, nasopharyngeal duct, hyperplasia	0	0	2 (1.0)	4* (1.5)
Epithelium, nasopharyngeal duct, necrosis	0	0	0	2 (2.0)
Epithelium, nasopharyngeal duct, regeneration	0	0	2 (1.5)	2 (3.5)
Lamina propria, mineralization	0	33** (1.4)	34** (1.4)	44** (1.5)
Lamina propria, pigmentation	0	4 (1.3)	11** (1.6)	25** (2.4)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Nasopharyngeal duct, inflammation, suppurative	0	6* (1.5)	8** (2.0)	20** (1.9)
Nerve, atrophy	0	33** (1.7)	44** (2.0)	44** (2.5)
Olfactory epithelium, atrophy	1 (1.0)	38** (1.8)	41** (2.0)	41** (2.0)
Olfactory epithelium, hyperplasia, basal cell	0	1 (1.0)	9** (1.0)	28** (1.8)
Olfactory epithelium, metaplasia, respiratory	4 (1.3)	40** (2.2)	43** (2.4)	47** (2.9)
Olfactory epithelium, necrosis	1 (2.0)	3 (2.0)	0	12** (2.0)
Olfactory epithelium, pigmentation	6 (1.7)	18** (1.6)	12 (1.3)	21** (1.5)
Olfactory epithelium, squamous metaplasia	0	0	1 (1.0)	4* (1.3)
Respiratory epithelium, atrophy	0	2 (1.5)	5* (1.4)	6** (1.5)
Respiratory epithelium, degeneration	0	0	0	3 (2.0)
Respiratory epithelium, metaplasia, squamous	0	1 (1.0)	3 (1.3)	7** (1.6)
Respiratory epithelium, necrosis	0	0	0	4* (2.8)
Respiratory epithelium, pigmentation	2 (1.0)	6 (1.2)	7 (1.1)	7* (1.3)
Turbinate, deformity	0	16**	22**	35**
Turbinate, hyperostosis	0	18** (1.5)	27** (1.9)	40** (2.2)
Female				
Three-month interim evaluation				
Number examined microscopically	10	–	–	10
Inflammation, suppurative	0	–	–	1 (2.0)
Inflammation, acute	0	–	–	1 (1.0)
Epithelium, nasopharyngeal duct, degeneration	0	–	–	1 (2.0)
Epithelium, nasopharyngeal duct, regeneration	0	–	–	1 (2.0)
Lamina propria, pigmentation	0	–	–	1 (1.0)
Nasopharyngeal duct, inflammation, acute	0	–	–	1 (1.0)
Nerve, atrophy	0	–	–	1 (1.0)
Olfactory epithelium, atrophy	0	–	–	2 (1.5)
Olfactory epithelium, metaplasia, respiratory	1 (1.0)	–	–	4 (1.0)
Olfactory epithelium, pigmentation	0	–	–	4* (1.0)
Turbinate, deformity	0	–	–	1
Two-year study				
Number examined microscopically	49	49	50	49
Foreign body	3	2	4	8**
Inflammation, suppurative	5 (1.2)	3 (2.0)	17** (1.5)	35** (2.1)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Epithelium, nasopharyngeal duct, degeneration	0	0	1 (3.0)	3* (2.7)
Epithelium, nasopharyngeal duct, hyperplasia	0	1 (1.0)	4* (1.0)	1 (2.0)
Epithelium, nasopharyngeal duct, necrosis	0	1 (1.0)	2 (3.5)	7** (3.0)
Epithelium, nasopharyngeal duct, regeneration	0	0	0	8** (3.1)
Lamina propria, mineralization	3 (1.0)	23** (1.1)	30** (1.4)	22** (1.5)
Lamina propria, pigmentation	1 (1.0)	0	6* (1.2)	14** (2.0)
Nasopharyngeal duct, inflammation, suppurative	0	2 (1.0)	5* (1.2)	15** (2.3)
Nerve, atrophy	0	38** (1.6)	41** (2.1)	38** (2.3)
Olfactory epithelium, atrophy	2 (1.5)	35** (1.6)	42** (1.7)	34** (1.8)
Olfactory epithelium, hyperplasia, basal cell	0	0	8** (1.1)	20** (1.7)
Olfactory epithelium, metaplasia, respiratory	1 (1.0)	42** (2.1)	43** (2.7)	36** (3.0)
Olfactory epithelium, necrosis	0	3 (1.7)	1 (2.0)	18** (1.8)
Olfactory epithelium, pigmentation	0	11** (1.0)	7** (1.3)	5** (1.2)
Olfactory epithelium, squamous metaplasia	0	2 (1.0)	1 (1.0)	5** (1.4)
Respiratory epithelium, atrophy	0	8** (1.5)	9** (1.7)	3* (1.0)
Respiratory epithelium, metaplasia, squamous	1 (1.0)	1 (1.0)	1 (1.0)	4* (1.5)
Respiratory epithelium, necrosis	0	1 (2.0)	2 (1.5)	17** (2.4)
Respiratory epithelium, pigmentation	0	1 (1.0)	5* (1.2)	5** (1.0)
Respiratory epithelium, regeneration	0	0	0	2 (2.0)
Turbinate, deformity	0	6*	20**	15**
Turbinate, hyperostosis	0	18** (1.3)	32** (1.7)	36** (2.2)

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test (interim evaluation) or the Poly-3 test (2-year study).
**$P \leq 0.01$.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Suppurative inflammation was predominantly characterized by accumulation of neutrophils within the nasal lumen. Acute inflammation was characterized by small accumulations of neutrophils within the epithelium with no appreciable luminal exudate. Epithelial hyperplasia in the nasopharyngeal duct was characterized by increased height and nuclear layers within the epithelium often with epithelial infoldings/invaginations. Necrosis of the nasopharyngeal duct epithelium was characterized by hypereosinophilia and cell swelling, and often there were large sections of sloughed necrotic epithelium. Acute or suppurative inflammation of the nasopharyngeal duct were characterized by small accumulations of intraepithelial neutrophils or accumulation of neutrophils within the lumen, respectively. Regeneration of the nasopharyngeal

duct epithelium was characterized by multiple layers of undifferentiated cells that lacked cilia. Degeneration of the nasopharyngeal duct epithelium was characterized by lack of goblet cells and ciliated brush border in the affected tissue. Lamina propria mineralization was characterized by focal accumulation of pale, basophilic, poorly laminated material within the basal lamina of both respiratory and olfactory epithelium and was similar to that seen at 2 years. Atrophy of the olfactory epithelium was characterized by thinning of the epithelial layers. Respiratory metaplasia of the olfactory epithelium was characterized by the replacement of the olfactory epithelium with respiratory epithelium often accompanied by loss of the underlying Bowman's glands. Nerve atrophy was always associated with atrophy of the olfactory epithelium, though not all animals with epithelial atrophy had corresponding atrophy of the underlying nerve bundles. Necrosis of the olfactory epithelium was characterized by hypereosinophilia and cell swelling and often there were large sections of sloughed necrotic epithelium. Pigmentation of the olfactory epithelium, nasopharyngeal duct epithelium, or lamina propria was characterized by the presence of round cells, likely macrophages, with abundant intracytoplasmic golden brown pigmented material. Squamous metaplasia of the respiratory epithelium was characterized by replacement of small sections of normal resident respiratory epithelium by a multilayer epithelium that appeared to have extensive intracellular connections, a flattened luminal layer, and evidence, though typically minimal, of keratin production. Deformity of the turbinates was characterized by a wide spectrum of abnormally shaped turbinates and fusion of turbinates to the nasal septum, nasal wall, or each other.

The lesions in the nose of the 1,000 mg/kg Wistar Han rats were similar in character to those observed in the 3-month study in F344/NTac rats, although the incidences of the lesions were slightly lower in the Wistar Han rats.

At 2 years, numerous nasal lesions were increased in incidence in dosed males and/or females, relative to the vehicle controls (Table 11, Table A-3, and Table B-3). Lesions in the nasal lumen included increased incidences of suppurative inflammation in male and female rats and foreign material in female rats. Suppurative inflammation was characterized by accumulations of, predominantly, neutrophils within the lumen of the nasal passages. The inflammatory infiltrate in general was most prominent in Level III (the most posterior level) of the nose, though in more severely affected animals, the neutrophilic exudate extended into Level II and occasionally Level I (the most rostral level). Rarely, there was an inflammatory exudate in Level I without inflammation with the more posterior levels, and in these animals, the inflammation was almost always associated with the presence of foreign material. In addition to the luminal exudate, there were variable numbers of neutrophils undergoing transepithelial migration and present in the underlying submucosal stroma. Some animals also had infiltrates of lymphocytes and plasma cells within the subepithelial stroma. The presence of foreign bodies within the nasal lumen consisted most often of pieces of plant material or hair shafts and occasionally other small refractile pieces of unidentifiable material. Typically, the foreign material was associated with a neutrophilrich luminal exudate.

Increased incidences of lesions of the nasopharyngeal duct of dosed groups included degeneration, hyperplasia, necrosis, and regeneration of the epithelium and suppurative inflammation within the duct itself. Necrosis was characterized by hypereosinophilia and cell swelling and often there were large sections of sloughed necrotic epithelium. Hyperplasia was characterized by increased height and nuclear layers within the epithelium often with epithelial infoldings or invaginations. Degeneration was characterized by epithelium that exhibited

evidence of damage (e.g., cellular vacuolation and/or swelling) but was not to the point of necrosis. Regeneration was comparable to the aspect of this lesion seen in the respiratory epithelium. Suppurative inflammation in the nasopharyngeal duct was characterized by exudation of neutrophils within the nasopharyngeal duct lumen (Figure 12).

Changes within the lamina propria of dosed rats consisted of pigmentation and mineralization. Pigmentation was characterized by variably sized clusters of histiocytes with abundant intracytoplasmic accumulation of golden to greenish brown pigmented material that were located in the lamina propria generally associated with regions of bony proliferation/remodeling. Mineralization was associated with the basement membrane of both olfactory and respiratory epithelium, most commonly at Level III, but occasionally in Level II as well. Larger foci consisted of pale irregular laminated material. Enlarged deposits of the mineralized material usually protruded into the lamina propria, but occasionally projected outward into the epithelium and rarely protruded into the nasal lumen.

Lesions that increased in incidence in the olfactory epithelium of dosed rats included atrophy, necrosis, basal cell hyperplasia, respiratory and squamous metaplasia, and pigmentation. The olfactory epithelial lesions primarily were seen in Level III, but also affected the dorsal portion of Level II. Atrophy of the nerves underlying the olfactory epithelium was also significantly increased in all dosed groups of male and female rats at the 2-year evaluation.

Olfactory epithelium atrophy was characterized by focal to focally extensive regions of decreased numbers and disorganization of the nuclear layers and loss of the luminal eosinophilic border. Nerve atrophy was always associated with atrophy of the olfactory epithelium, though not all animals with epithelial atrophy had corresponding atrophy of the underlying nerve bundles. Olfactory epithelium necrosis was characterized by a segment or cluster of epithelial cells that were shrunken and hypereosinophilic with karyorrhexis and/or nuclear pyknosis. Olfactory basal cell hyperplasia was characterized by increased cell size and cell number of the basal cells of the olfactory epithelium, generally two to three cell layers in thickness, with the occasional formation of gland-like structures and occasionally extending down ducts into the underlying Bowman's glands. This lesion was not limited to the dorsal meatus as described in the 3-month F344/NTac rat study, but instead was often present in any/all remaining olfactory epithelium in affected animals. Metaplasia was characterized by partial to complete loss and replacement of the olfactory epithelium predominantly by respiratory epithelium and rarely by squamous epithelium. Occasionally, the metaplastic respiratory epithelium formed gland-like invaginations into the underlying stroma. The stroma underlying the affected olfactory epithelium was altered in that there was a loss of Bowman's glands and atrophy of the olfactory nerve bundles. Pigmentation of the olfactory epithelium was characterized by variable numbers of cells within the olfactory epithelium with abundant intracytoplasmic accumulation of golden brown pigmented material. These cells were most often present in regions of the epithelium that had atrophied or had evidence of prior damage and repair.

Significantly increased incidences of lesions in the respiratory epithelium included atrophy, necrosis, squamous metaplasia, and pigmentation. Degeneration of the respiratory epithelium was increased in the 1,000 mg/kg males, and regeneration of the respiratory epithelium was increased in the 1,000 mg/kg females, but neither of these increases was significant. The respiratory epithelium lesions were predominantly noted in Level III of the nose, with less severe lesions occasionally present in Level II and few lesions present in Level I. Atrophy of the

respiratory epithelium was characterized by flattening of the respiratory epithelium to a low cuboidal epithelium instead of the normal tall columnar epithelium. Degeneration of the respiratory epithelium was characterized by epithelium exhibiting evidence of damage (e.g., cellular vacuolation and swelling) but was not to the point of necrosis. Necrosis was characterized by hypereosinophilia and swelling of the respiratory epithelium and often there were large sections of affected epithelium sloughing off of an underlying basal epithelial layer. Regeneration was characterized by the presence of multiple layers of undifferentiated cells that lacked cilia in a region normally occupied by respiratory epithelium. Squamous metaplasia was characterized by the presence of regions in which the normal ciliated columnar epithelium had been replaced by squamous epithelium. Pigmentation was characterized by variable numbers of cells within the respiratory epithelium with abundant intracytoplasmic accumulation of golden brown pigmented material.

Changes involving the turbinates consisted of hyperostosis and deformity, which are considered to reflect postinflammation bony remodeling. These turbinate changes were most commonly seen in and most severe in Level III of the nose. These lesions were characterized by increased bony deposition (hyperostosis) and malformation (Figure 13). Occasional fusion of turbinates to the nasal septum and to each other was noted.

Lung: Compared to the vehicle controls at 2 years, the incidences of suppurative inflammation were significantly increased in 1,000 mg/kg males and females [males: vehicle control, 0/50; 100 mg/kg, 1/50 (2.0); 300 mg/kg, 3/50 (3.7); 1,000 mg/kg, 10/50 (3.7); females: 1/50 (2.0), 3/49 (2.0), 2/50 (3.5), 9/48 (3.4); Table A-3 and Table B-3]; the severity was increased at 300 and 1,000 mg/kg. Suppurative inflammation of moderate to marked severity was characterized by acute accumulation of massive numbers of neutrophils and fibrin within alveolar spaces and bronchial and bronchiolar lumens. These lesions often were focally extensive over large portions of the affected lung lobes. There was occasionally necrosis of alveolar septa and bronchial and bronchiolar epithelium and focal or multifocal hemorrhage. The findings were considered consistent with aspiration pneumonia, though no foreign material was evident within the examined sections.

Heart: Epicardium inflammation was seen at 2 years in 300 and 1,000 mg/kg males [vehicle control, 0/50; 100 mg/kg, 0/50; 300 mg/kg, 1/50 (1.0); 1,000 mg/kg, 5/50 (2.2); Table A-3] and in all dosed groups of females [0/50, 2/48 (2.0), 2/50 (2.0), 4/48 (1.5); Table B-3]; the incidences at 1,000 mg/kg were significantly greater than the vehicle control incidences. A single incidence of pericardium inflammation of marked severity was seen in a 1,000 mg/kg male. Inflammation of the pericardium or epicardium occasionally extended into the underlying heart and was an extension of the inflammatory process in the lungs. Three incidences of mild myocardium necrosis occurred in the 1,000 mg/kg males. Myocardial necrosis was diagnosed when there were multiple cardiomyocytes that were swollen and hypereosinophilic with a loss of cellular and nuclear detail. These cells often had an associated predominantly neutrophilic inflammatory infiltrate. This lesion was distinguished from cardiomyopathy due to the presence of multiple cardiomyocytes in the same acute stage of cellular death.

Bone Marrow: Incidences of bone marrow hyperplasia were significantly increased in all dosed groups of females at 2 years [6/50 (1.7), 14/50 (2.1), 16/50 (2.4), 13/50 (2.5); Table B-3]. The hyperplastic bone marrow was predominantly composed of immature cells of granulocyte lineage.

Spleen: The incidences of lymphoid depletion at 2 years were significantly increased in the 1,000 mg/kg males and all dosed groups of females [males: 1/50 (2.0), 2/50 (2.0), 1/50 (1.0), 13/50 (2.2); females: 0/50, 7/49 (1.7), 5/48 (1.8), 17/43 (1.7); Table A-3 and Table B-3]. Lymphoid depletion consisted of a decrease in white pulp (periarteriolar, follicular, or multizonal) compared to that in the vehicle control rats.

Other Organs: Uterine endometrial adenocarcinoma was observed in all dosed groups of females in the original evaluation (3/50, 9/50, 5/50, 4/50; Table B-1 and Table B-2); there was no statistically significant difference between the groups. A longitudinal evaluation of the residual uterine tissue revealed numerous uterine neoplasms in all groups, as well as occurrences of atypical hyperplasia (Table B-4). When the incidences of uterine neoplasms from the original evaluation and the longitudinal evaluation were combined, there was not considered to be an effect due to green tea extract exposure.

Mice

Three-month Study

Six males and four females administered 1,000 mg/kg died before the end of the study (Table 12). Early deaths were due to liver necrosis. Final mean body weights and mean body weight gains of males administered 250 mg/kg or greater and females administered 125 mg/kg or greater were significantly less than those of the vehicle controls (Table 12 and Figure 7). Clinical findings included lethargy, abnormal breathing, and ataxia in early death females and ruffled fur in two surviving 1,000 mg/kg males.

Table 12. Survival and Body Weights of Mice in the Three-month Gavage Study of Green Tea 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	24.4 ± 0.3	40.7 ± 1.0	16.3 ± 0.9	–
62.5	10/10	24.2 ± 0.4	38.5 ± 1.1	14.3 ± 1.0	95
125	10/10	24.1 ± 0.3	39.4 ± 1.0	15.3 ± 0.9	97
250	10/10	24.3 ± 0.4	35.4 ± 0.9**	11.1 ± 0.8**	87
500	10/10	24.3 ± 0.3	34.1 ± 0.9**	9.8 ± 0.8**	84
1,000	4/10[c]	24.2 ± 0.3	30.8 ± 0.9**	6.3 ± 0.6**	76
Female					
0	10/10	18.7 ± 0.2	30.4 ± 0.6	11.7 ± 0.6	–
62.5	10/10	18.9 ± 0.3	32.9 ± 1.1	14.0 ± 0.9	92
125	10/10	18.3 ± 0.2	26.6 ± 0.7**	8.3 ± 0.6**	88
250	10/10	18.6 ± 0.2	26.8 ± 0.7**	8.2 ± 0.6**	88
500	10/10	18.6 ± 0.2	24.3 ± 0.4**	5.7 ± 0.5**	80
1,000	6/10[d]	18.3 ± 0.2	26.2 ± 0.3**	7.6 ± 0.2**	86

**Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test.
[a]Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b]Number of animals surviving at 14 weeks/number initially in group.
[c]Weeks of deaths: 1, 1, 1, 1, 1, 8.
[d]Weeks of deaths: 1, 1, 10, 11.

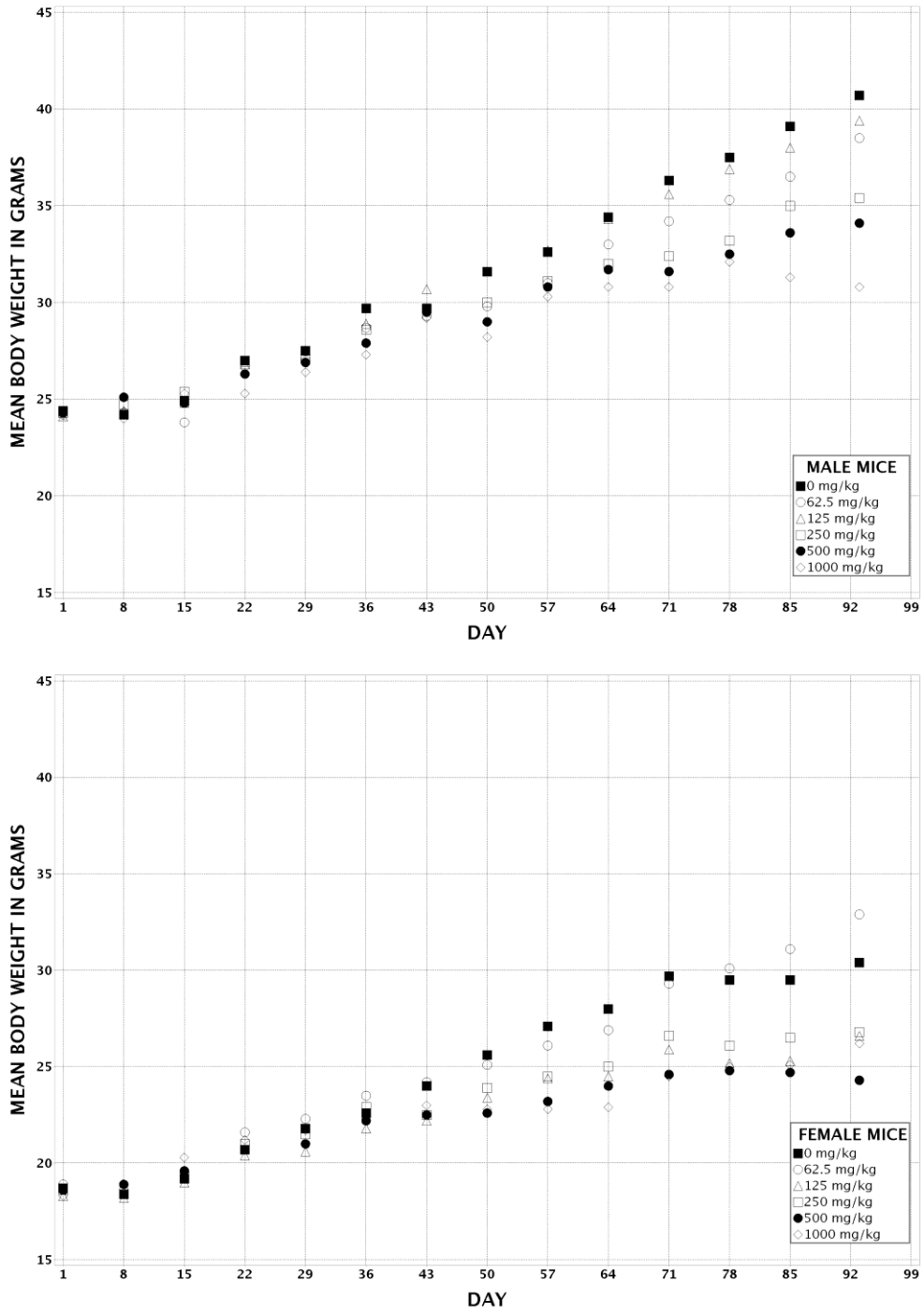

Figure 7. Growth Curves for Mice Administered Green Tea Extract by Gavage for Three Months

No changes in the hematology data for mice were considered attributable to the administration of green tea extract (Table F-2).

The absolute thymus weight of 1,000 mg/kg males was significantly less by approximately 35% and those of 500 and 1,000 mg/kg females were significantly less (up to 23%) than those of the vehicle control groups (Table G-3). The changes in the thymus weights were most likely due to

stress. Other statistically significant changes in organ weights were not considered biologically significant or were considered related to changes in body weight.

Males administered 500 mg/kg exhibited a significantly decreased spermatid per testis count (11%) compared to vehicle controls (Table H-3). Females administered 500 mg/kg spent significantly more time in extended diestrus than did the vehicle controls (Table H-4). Thus, under the conditions of these studies, green tea extract administered by oral gavage exhibited the potential to be a reproductive toxicant in male and female B6C3F1/N mice.

In the liver, increased incidences of centrilobular necrosis, glycogen depletion, pigmentation, mitosis, karyomegaly, and chronic inflammation (females) occurred in the 1,000 mg/kg groups of male and female mice (Table 13). Increased incidences of glycogen depletion in 250 and 500 mg/kg males and females and centrilobular necrosis in 125 mg/kg males were also observed. The liver necrosis in severely affected animals was characterized by a centrilobular to panlobular necrosis and hemorrhage. In the livers with minimal to mild necrosis, there were more individualized hepatocytes undergoing necrosis with minimal to no inflammation. If inflammatory cells were present, they were usually macrophages, lymphocytes, and plasma cells. Hepatocytes were characterized by pyknotic, condensed nuclei and shrunken eosinophilic cytoplasm. Chronic inflammation consisted of infiltration of mononuclear cells in the areas of hepatocellular necrosis. Mitosis was characterized by the presence of hepatocytes with mitotic figures in the intervening region between the centrilobular and periportal regions. Accumulation of golden brown cytoplasmic pigment was present in the Kupffer cells. Special stains were not performed on mouse liver sections. However, a similar pigment was found in the rat livers as a treatment-related effect. Minimal karyomegaly was diagnosed when 10 to 50 enlarged nuclei were observed per section. Diagnosis was not made if there was significant concurrent hepatocyte damage or cell swelling due to necrosis or fatty change. The karoymegalic hepatocytes occurred in mice having minimal levels of necrosis, and were located in the midzonal regions between centrilobular and periportal areas. The nuclei were 2.5 to 3 times normal size and had clumped chromatin or intranuclear vacuoles, which most likely represented invagination of the nuclear membrane. Rare hepatocytes with three nuclei were also present.

Significant increases in the incidences of lesions of the nose occurred in the 500 and 1,000 mg/kg groups of male and female mice and included nerve atrophy and atrophy, metaplasia, and necrosis (1,000 mg/kg females) of the olfactory epithelium (Table 13). Incidences of nerve atrophy and olfactory epithelium atrophy and metaplasia were also significantly increased in 250 mg/kg males. The incidence of hyaline droplet accumulation of the respiratory epithelium was significantly increased in 500 mg/kg females. Other biologically important lesions that were not significantly increased included inflammation; hyperplasia of the glands of the olfactory epithelium; degeneration of the nasopharyngeal duct (females); basal cell hyperplasia and necrosis (males) of the olfactory epithelium; and hyperplasia, squamous metaplasia, and necrosis of the respiratory epithelium. Several of these lesions that lacked statistical significance in the 3-month study were significantly increased in incidence in the 2-year study. These varying lesions are most likely all part of a spectrum of changes associated with the same pathologic process.

Lesions of the olfactory epithelium were focal to locally extensive and frequently bilateral and/or involved multiple levels (Levels II and III). Olfactory epithelium atrophy was associated with thinning of the layers of olfactory epithelial cells, especially in the dorsal meatus, compared to

vehicle controls. Nerve atrophy was noted by the loss of olfactory nerve bundles in the submucosal regions underlying the olfactory epithelium in the dorsal meatus of Level II and nasal turbinates of Level III. Atrophy was most notable in the dorsal and dorsolateral meatus. Nerve atrophy was frequently accompanied by olfactory epithelium metaplasia characterized by the transition of olfactory epithelium to respiratory epithelium.

Olfactory epithelium necrosis was characterized by loss of olfactory sensory epithelial cells or pyknosis and karyorrhexis of olfactory epithelial nuclei with infiltrates of neutrophils and lesser numbers of lymphocytes and plasma cells.

Table 13. Incidences of Selected Nonneoplastic Lesions in Mice in the Three-month Gavage Study of Green Tea 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
Depletion Glycogen[b]	2 (1.0)[c]	0	2 (1.5)	8* (1.5)	10** (1.7)	4 (2.5)
Karyomegaly	0	0	0	0	0	2 (1.0)
Mitosis	0	0	0	0	0	3 (1.3)
Pigmentation	0	0	0	0	0	2 (1.0)
Centrilobular Necrosis	0	0	2 (1.0)	0	0	8** (3.1)
Nose	10	10	10	10	10	10
Inflammation	0	0	1 (3.0)	0	1 (2.0)	1 (2.0)
Glands, Olfactory Epithelium, Hyperplasia	0	0	0	0	0	3 (1.3)
Nerve, Atrophy	0	0	0	5* (1.2)	7** (1.1)	5* (1.8)
Olfactory Epithelium, Atrophy	0	1 (1.0)	0	4* (1.3)	4* (1.8)	4* (1.3)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	0	0	0	0	3 (2.3)
Olfactory Epithelium, Metaplasia	0	0	0	5* (1.0)	5* (1.2)	5* (2.2)
Olfactory Epithelium, Necrosis	0	0	1 (3.0)	0	1 (3.0)	3 (1.7)
Respiratory Epithelium, Hyperplasia	0	0	0	0	0	3 (1.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	0	1 (2.0)
Respiratory Epithelium, Necrosis	0	0	0	0	1 (4.0)	1 (2.0)
Lymph Node, Mesenteric	10	10	10	10	10	10
Atrophy	4 (1.0)	0*	0*	0*	2 (1.0)	8 (1.4)
Lymph Node, Mandibular	10	10	10	10	10	10

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Atrophy	1 (1.0)	0	0	0	2 (1.0)	7** (1.9)
Thymus	10	10	10	10	10	10
Atrophy	0	0	0	0	0	6** (3.3)
Intestine, Large, Colon	10	0	0	0	10	10
Peyer's Patch, Atrophy	0	–	–	–	0	1 (1.0)
Intestine, Small, Ileum	10	0	0	0	10	10
Peyer's Patch, Atrophy	0	–	–	–	0	2 (2.0)
Female						
Liver	10	10	10	10	10	10
Depletion Glycogen	0	0	0	1 (1.0)	4* (1.5)	7** (2.1)
Inflammation, Chronic	0	0	0	0	0	3 (2.0)
Karyomegaly	0	0	0	0	0	5* (1.0)
Mitosis	0	0	0	0	0	2 (2.0)
Pigmentation	0	0	0	0	0	2 (1.0)
Centrilobular Necrosis	0	0	0	0	0	7** (2.4)
Nose	10	10	10	10	10	10
Inflammation	0	1 (2.0)	1 (2.0)	0	0	1 (1.0)
Glands, Olfactory Epithelium, Hyperplasia	0	0	0	0	0	1 (1.0)
Nasopharyngeal Duct, Degeneration	0	0	0	0	0	1 (1.0)
Nerve, Atrophy	0	0	1 (1.0)	1 (1.0)	7** (1.3)	5* (1.4)
Olfactory Epithelium, Atrophy	0	0	1 (2.0)	0	4* (1.0)	4* (1.8)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	0	0	0	0	1 (2.0)
Olfactory Epithelium, Metaplasia	0	0	1 (1.0)	1 (1.0)	7** (1.6)	6** (1.3)
Olfactory Epithelium, Necrosis	0	0	0	0	1 (1.0)	4* (1.5)
Respiratory Epithelium, Hyaline Droplet	0	0	0	1 (2.0)	4* (1.8)	0
Respiratory Epithelium, Hyperplasia	0	0	0	0	0	2 (2.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	0	1 (1.0)
Respiratory Epithelium, Necrosis	0	0	0	0	0	1 (3.0)
Spleen	10	10	10	10	10	10

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Atrophy, Lymphoid	0	0	0	1 (1.0)	4* (1.0)	4* (2.8)
Lymph Node, Mesenteric	10	10	10	10	10	9
Atrophy	2 (1.0)	0	0	0	2 (1.5)	3 (2.3)
Lymph Node, Mandibular	10	10	10	10	10	10
Atrophy	0	0	0	2 (1.0)	2 (1.5)	4* (2.0)
Thymus	10	10	10	10	10	10
Atrophy	0	0	0	0	0	4* (3.3)
Intestine, Large, Cecum	10	0	0	0	0	10
Peyer's Patch, Atrophy	0	–	–	–	–	1 (1.0)
Intestine, Small, Ileum	10	0	0	0	0	10
Peyer's Patch, Atrophy	0	–	–	–	–	2 (2.5)

*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.

Basal cell hyperplasia of the olfactory epithelium was sporadically seen in 1,000 mg/kg males and females and was located in the dorsal meatus of Levels II and III. There were increased numbers of basal cells and instead of a single layer of basophilic nuclei, nuclei were frequently piled up or crowded and extended up into the olfactory cells. Occasionally, hyperplastic cuboidal cells extended to the Bowman's glands and appeared as nodular proliferations of cells underlying the olfactory epithelium. The proliferating basal cells frequently obscured the lumen of the Bowman's glands.

Inflammation in the nose was an infrequent finding in mice compared to rats. Inflammation was characterized by the presence of infiltrates of neutrophils with lesser numbers of lymphocytes and plasma cells in the lamina propria of the respiratory epithelium in various locations but most frequently along the septum or lateral walls and dorsal meatus (Level I) and in some cases the lamina propria of the olfactory epithelium (Levels II and III). Occasionally this mixed cellular infiltrate would extend into the overlying epithelium (either respiratory or olfactory, depending on the location).

The nasopharyngeal duct located at Level III was degenerated in one 1,000 mg/kg female. This lesion was characterized by loss of goblet cells and occasionally the attenuation of the typical tall columnar epithelium.

Squamous metaplasia of the respiratory epithelium in Levels I and II of the nasal cavity was characterized by transformation of the columnar cuboidal ciliated respiratory epithelium to well-differentiated stratified squamous epithelium intermittently along the lateral wall and a portion of nasal septum and dorsolateral meatus. This lesion occurred in association with respiratory epithelium hyperplasia, which was characterized by the proliferation of nonciliated cuboidal epithelium that affected extensive areas of the respiratory epithelium at Levels I and II along the nasal septum and lateral walls of the nasal cavity. Respiratory epithelium necrosis was

characterized by loss of respiratory epithelial cells, or pyknosis and karyorrhexis of respiratory epithelial nuclei, and sloughing of necrotic cells admixed with infiltrates of degenerative and viable neutrophils and lesser numbers of lymphocytes and plasma cells and cell debris.

Evidence of lymphoid tissue atrophy was seen in the spleen (females), lymph nodes (mesenteric and mandibular), thymus, and intestine (Peyer's patches) (Table 13). These lesions may represent direct toxicity, but it is likely they are evidence of an indirect effect, such as stress.

In the spleen, dose-related increased incidences and severities of lymphoid atrophy were noted in 250 mg/kg or greater females (Table 13). Atrophy in the spleen was characterized by loss of B cell lymphocytes in the germinal centers and follicles and loss of T cell lymphocytes in the periarteriolar lymphoid sheath region.

Increased incidences of mesenteric lymphoid atrophy occurred in 1,000 mg/kg males and females, with the males being more frequently affected, and increased incidences of mandibular lymphoid atrophy occurred in 500 and 1,000 mg/kg males and 250 mg/kg or greater females (Table 13). The severity of these lesions generally increased with increasing dose. In the mesenteric lymph node, atrophy of the T lymphocytes in the paracortex and B cells of the follicles was characterized by loss of lymphocytes and apoptosis of lymphocytes. Apoptotic lymphocytes had condensed nuclear chromatin and cytoplasm, pyknosis, and karyorrhexis. In the mandibular lymph node, atrophy of the T lymphocytes in the paracortex and B cells of the follicles was characterized by loss of lymphocytes, lymphocytes with condensed and fragmented nuclear chromatin (pyknosis and karyorrhexis), and shrunken cells with condensed cytoplasm.

Mild to marked thymic atrophy occurred in 1,000 mg/kg males and females (Table 13). Atrophy was characterized by variable thinning of the cortex due to loss of T cells by apoptosis.

Sporadic cases of minimal to moderate atrophy of the Peyer's patches were seen in the ileum and cecum or colon of 1,000 mg/kg males and females (Table 13). This lesion was characterized by lymphoid depletion and apoptosis of, largely, B lymphocytes residing in the follicle and occasional T lymphocytes in the perifollicular areas.

Dose Selection Rationale: Significant decreases in survival were observed in the 1,000 mg/kg mice. Treatment-related decreases in body weight gain in the 500 mg/kg groups were considered dose limiting. Therefore, the green tea extract doses selected for the 2-year gavage study in mice were 30, 100, and 300 mg/kg.

Two-year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 14 and in the Kaplan-Meier survival curves (Figure 8). Survival of dosed groups was similar to that of the vehicle control groups.

Body Weights and Clinical Findings

Mean body weights of 100 and 300 mg/kg males were at least 10% less than those of the vehicle control group after weeks 89 and 65, respectively, and mean body weights of 100 and 300 mg/kg females were at least 10% less after weeks 25 and 17, respectively (Figure 9; Table 15 and Table 16). There were no clinical findings related to green tea extract administration.

Table 14. Survival of Mice in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	0	0	2
Moribund	6	8	7	10
Natural deaths	11	6	10	1
Animals surviving to study termination	33	36	33[b]	37
Percent probability of survival at end of study[c]	66	72	66	77
Mean survival (days)[d]	694	702	705	699
Survival analysis[e]	P = 0.325N	P = 0.657N	P = 1.000	P = 0.296N
Female				
Animals initially in study	50	50	50	50
Accidental deaths[a]	3	6	1	0
Moribund	10	2	2	6
Natural deaths	3	9	3	5
Animals surviving to study termination	34	33[f]	44	39
Percent probability of survival at end of study	72	75	90	78
Mean survival (days)	665	620	701	680
Survival analysis	P = 0.808N	P = 1.000	P = 0.061N	P = 0.779N

[a]Censored from survival analyses.
[b]Includes one animal that died during the last week of the study (censored from survival analysis).
[c]Kaplan-Meier determinations.
[d]Mean of all deaths (uncensored, censored, and terminal kill).
[e]The result of the life table trend test[106] is in the vehicle control column, and the results of the life table pairwise comparisons[105] with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by **N**.
[f]Includes one animal that died during the last week of the study (not censored from survival analysis).

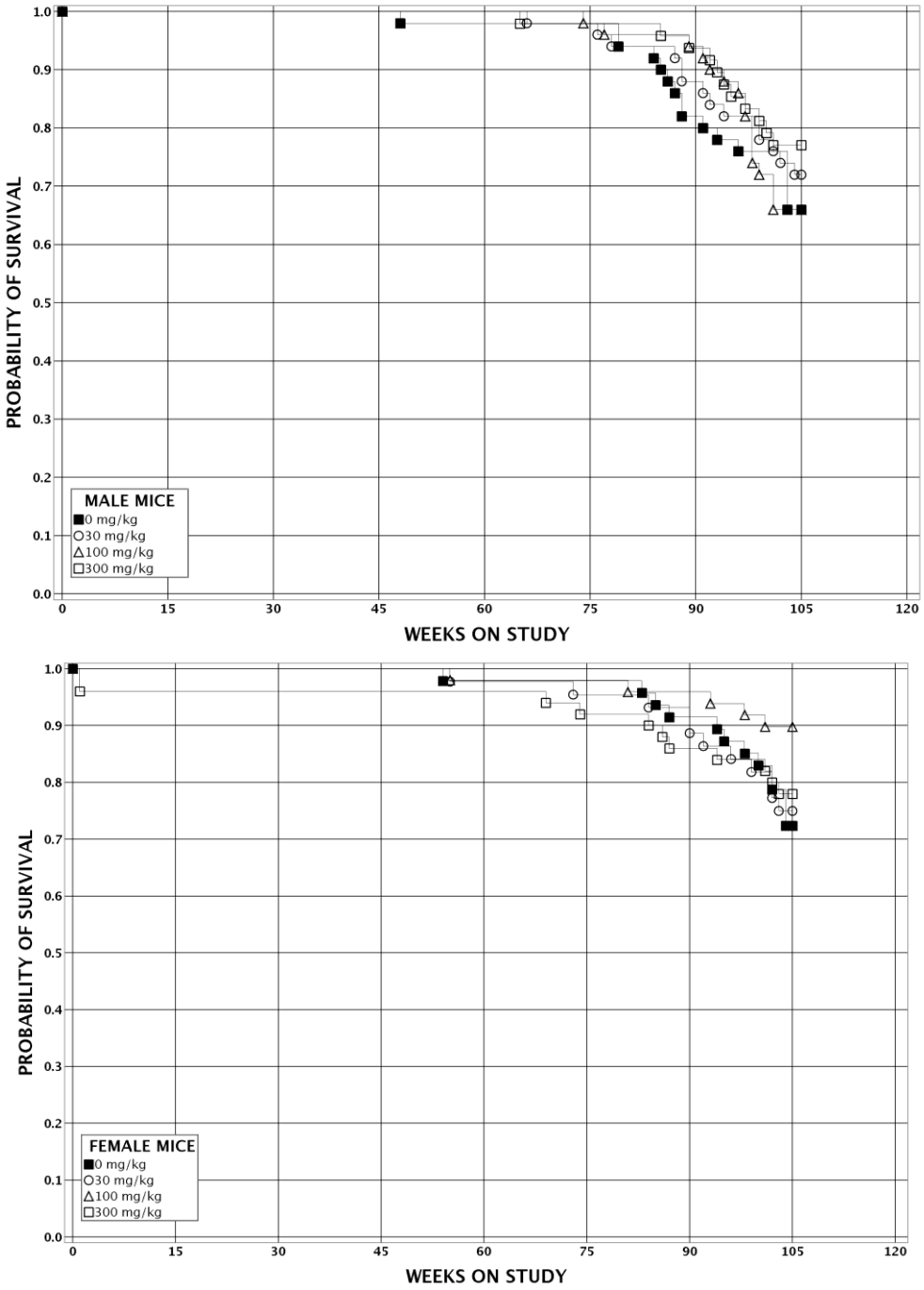

Figure 8. Kaplan-Meier Survival Curves for Mice Administered Green Tea Extract by Gavage for Two Years

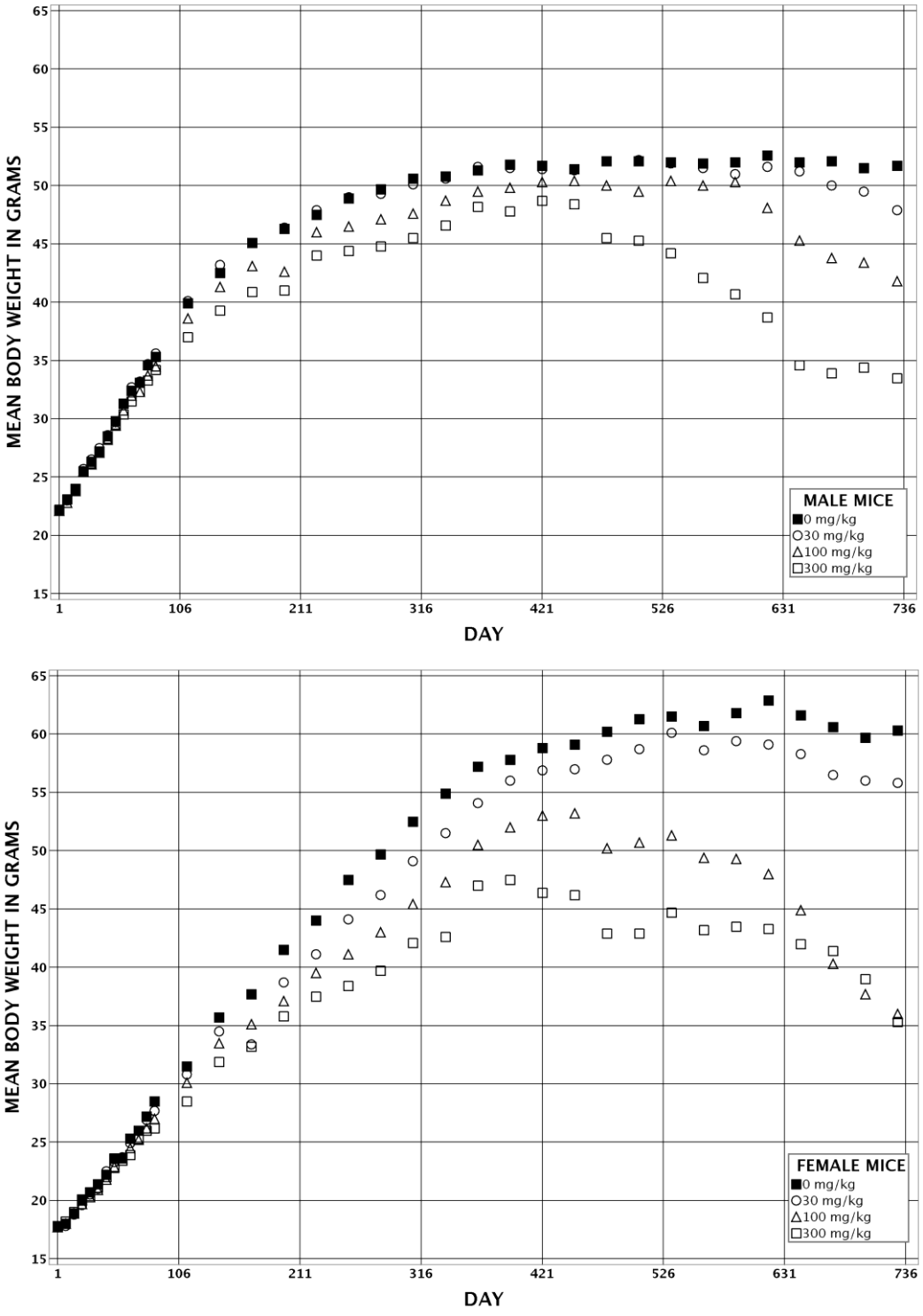

Figure 9. Growth Curves for Mice Administered Green Tea Extract by Gavage for Two Years

Table 15. Mean Body Weights and Survival of Male Mice in the Two-year Gavage Study of Green Tea Extract

Day	0 mg/kg		30 mg/kg			100 mg/kg			300 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	22.1	50	22.2	101	50	22.1	100	50	22.2	101	50
8	23.1	50	23.0	100	50	22.8	99	50	23.1	100	50
15	24.0	50	24.0	100	50	23.8	99	50	23.9	100	50
22	25.5	50	25.7	101	50	25.5	100	50	25.5	100	50
29	26.3	50	26.5	101	50	26.1	100	50	26.2	100	50
36	27.2	50	27.5	101	50	27.1	100	50	27.2	100	50
43	28.5	50	28.6	101	50	28.2	99	50	28.3	99	50
50	29.8	50	29.7	100	50	29.4	99	50	29.5	99	50
57	31.3	50	31.3	100	50	30.7	98	50	30.4	97	50
64	32.4	50	32.7	101	50	32.0	99	50	31.5	97	50
71	33.1	50	33.2	100	50	32.3	97	50	32.3	98	50
78	34.6	50	34.7	100	50	33.7	98	50	33.3	96	50
85	35.3	50	35.6	101	50	34.5	98	50	34.2	97	50
113	39.9	50	40.1	100	50	38.6	97	50	37.0	93	50
141	42.5	50	43.2	101	50	41.3	97	50	39.3	93	50
169	45.1	50	45.1	100	50	43.1	96	50	40.9	91	50
197	46.3	50	46.4	100	50	42.6	92	50	41.0	89	50
225	47.5	50	47.9	101	50	46.0	97	50	44.0	93	50
253	48.9	50	49.0	100	50	46.5	95	50	44.4	91	50
281	49.7	50	49.3	99	50	47.1	95	50	44.8	90	50
309	50.6	50	50.1	99	50	47.6	94	50	45.5	90	50
337	50.8	49	50.6	100	50	48.7	96	50	46.6	92	50
365	51.3	49	51.6	101	50	49.5	97	50	48.2	94	50
393	51.8	49	51.5	99	50	49.8	96	50	47.8	92	50
421	51.7	49	51.4	99	50	50.3	97	50	48.7	94	50
449	51.4	49	51.3	100	50	50.4	98	50	48.4	94	48
477	52.1	49	52.1	100	49	50.0	96	50	45.5	87	47
505	52.1	49	52.2	100	49	49.5	95	50	45.3	87	47
533	52.0	49	51.9	100	48	50.4	97	49	44.2	85	47
561	51.9	47	51.5	99	47	50.0	96	48	42.1	81	47
589	52.0	46	51.0	98	47	50.3	97	48	40.7	78	47
617	52.6	41	51.6	98	44	48.1	91	48	38.7	74	46
645	52.0	39	51.2	98	42	45.3	87	45	34.6	67	44
673	52.1	38	50.0	96	41	43.8	84	43	33.9	65	41
701	51.5	38	49.5	96	39	43.4	84	35	34.4	67	38
Mean for Weeks											
1–13	28.7	–	28.8	100	–	28.3	99	–	28.3	99	–
14–52	46.8	–	46.9	100	–	44.6	95	–	42.6	91	–
53–101	51.9	–	51.3	99	–	48.5	93	–	42.5	82	–

Table 16. Mean Body Weights and Survival of Female Mice in the Two-year Gavage Study of Green Tea Extract

Day	0 mg/kg		30 mg/kg			100 mg/kg			300 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	17.8	50	17.8	100	50	17.7	100	50	17.7	99	50
8	18.0	47	17.8	99	46	18.0	100	49	18.2	101	48
15	18.9	47	18.8	99	46	18.9	100	49	19.0	100	48
22	20.1	47	19.6	98	45	19.7	98	49	20.0	100	48
29	20.7	47	20.5	99	44	20.3	98	49	20.3	98	48
36	21.4	47	21.0	99	44	20.9	98	49	21.1	99	48
43	22.2	47	22.5	101	44	21.8	98	49	22.0	99	48
50	23.6	47	23.3	99	44	22.9	97	49	22.8	97	48
57	23.6	47	23.7	101	44	23.6	100	49	23.4	99	48
64	25.3	47	24.9	99	44	24.5	97	49	23.9	95	48
71	26.0	47	26.0	100	44	25.2	97	49	25.3	98	48
78	27.2	47	26.9	99	44	26.2	96	49	26.0	95	48
85	28.5	47	27.7	98	44	27.0	95	49	26.2	92	48
113	31.5	47	30.8	98	44	30.1	96	49	28.5	91	48
141	35.7	47	34.5	97	44	33.5	94	49	31.9	89	48
169	37.7	47	33.4	89	44	35.1	93	49	33.2	88	48
197	41.5	47	38.7	93	44	37.1	89	49	35.8	86	48
225	44.0	47	41.1	93	44	39.5	90	49	37.5	85	48
253	47.5	47	44.1	93	44	41.1	86	49	38.4	81	48
281	49.7	47	46.2	93	44	43.0	86	49	39.7	80	48
309	52.5	47	49.1	94	44	45.4	87	49	42.1	80	48
337	54.9	47	51.5	94	44	47.3	86	49	42.6	78	48
365	57.2	47	54.1	95	44	50.5	88	49	47.0	82	48
393	57.8	46	56.0	97	43	52.0	90	48	47.5	82	48
421	58.8	46	56.9	97	43	53.0	90	48	46.4	79	48
449	59.1	46	57.0	97	43	53.2	90	48	46.2	78	48
477	60.2	46	57.8	96	43	50.2	84	48	42.9	71	48
505	61.3	46	58.7	96	43	50.7	83	48	42.9	70	47
533	61.5	46	60.1	98	42	51.3	83	48	44.7	73	46
561	60.7	46	58.6	97	42	49.4	81	48	43.2	71	46
589	61.8	45	59.4	96	41	49.3	80	47	43.5	70	45
617	62.9	43	59.1	94	41	48.0	76	47	43.3	69	43
645	61.6	43	58.3	95	38	44.9	73	46	42.0	68	43
673	60.6	41	56.5	93	37	40.3	67	46	41.4	68	42
701	59.7	39	56.0	94	36	37.7	63	45	39.0	65	42
Mean for Weeks											
1–13	22.6	–	22.3	99	–	22.1	98	–	22.0	97	–
14–52	43.9	–	41.0	93	–	39.1	89	–	36.6	83	–
53–101	60.2	–	57.6	96	–	48.5	81	–	43.8	73	–

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the nose, lung, bone marrow, mandibular lymph node, liver, adrenal gland, and pancreatic islets. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix C for male mice and Appendix D for female mice.

Nose: The incidences of suppurative inflammation and foreign body (except 30 mg/kg females) in all dosed groups of mice were significantly greater than the vehicle control group incidences (Table 17, Table C-3, and Table D-3). Increased incidences of lumen pigmentation occurred in all dosed groups, and the increases were significant in 30 and 300 mg/kg females. The incidences of hyperostosis, septum perforation, and turbinate atrophy were significantly increased in 100 and 300 mg/kg mice. The severity of these lesions generally increased with increasing dose.

Inflammation of the nasal cavity was characterized by accumulations of primarily neutrophils within the nasal lumen. In addition, variable numbers of neutrophils were undergoing transepithelial migration, and neutrophils were present in the underlying submucosal stroma (Figure 14). The foreign body material ranged from large pieces of plant material to hair shafts and other small refractile pieces of unidentifiable material. The foreign bodies were typically surrounded by neutrophils. Acellular greenish-brown debris was also present within the lumen of the nasal cavity in some animals (Figure 15). A small number of animals had a similar pigmented material accumulated in intraluminal macrophages. Hyperostosis was variably characterized by deposition of woven and lamellar bone within the nasal septum and along the ventral surface of the flat bones lining the dorsal portion of the nasal cavity, primarily in Level III, but occasionally extending to Level II. Some animals with expanded nasal septa had enlarged and irregularly shaped bone marrow spaces within the bony deposits. Perforation of the septum in Level I consisted of a discontinuation of the septum separating the right and left nasal passages. Turbinate atrophy varied from thinned and/or slightly blunted nasoturbinates and maxilloturbinates of Levels I and II to pronounced blunting and fusion of the turbinates in Levels I and II and the ethmo-turbinates of Level III (Figure 16 and Figure 17).

Nasopharyngeal duct degeneration and inflammation occurred in 100 and 300 mg/kg mice, and inflammation was seen in one 30 mg/kg male (Table 17, Table C-3, and Table D-3). The increased incidence of degeneration in 300 mg/kg males was significant. One 300 mg/kg male had squamous metaplasia of the nasopharyngeal duct. Degeneration of the nasopharyngeal duct was characterized by cell swelling and vacuolation of the respiratory epithelium lining the duct. The inflammation and squamous metaplasia were similar to those previously described.

In the olfactory epithelium, incidences of atrophy in 30 and 100 mg/kg males and all dosed groups of females, respiratory metaplasia in all dosed groups, and fibrosis in 100 and 300 mg/kg males and females were significantly increased (Table 17, Table C-3, and Table D-3). The incidences of nerve atrophy were also significantly increased in all dosed groups. Necrosis occurred in a few mice administered 30 or 100 mg/kg. The lower incidence of olfactory epithelium atrophy in the high dose groups of males and females, when compared to the low and middle dose groups, may reflect a progression of some of the lesions past the point of atrophy and into fibrosis. Similarly, the few occurrences of necrosis of the olfactory epithelium probably

represent that this lesion was fairly early on in the process and was followed by atrophy, and subsequently fibrosis. Atrophy of the olfactory epithelium was seen in Level III and in the dorsal portion of Level II, and was associated with subsequent atrophy of the related olfactory nerve bundles. Olfactory epithelium atrophy ranged from focally extensive lesions of decreased number and disorganization of the nuclear layers and loss of the lumenal eosinophilic border to extensive thinning and cellular loss of the olfactory epithelium. When becoming metaplastic, the olfactory epithelium was replaced by respiratory epithelium. In some animals with respiratory metaplasia of the olfactory epithelium, there were variable numbers of invaginations of the respiratory epithelium resembling glands within the subepithelial stroma. When fibrosis was present, the stroma underlying the affected olfactory epithelium was altered in that there was a loss of Bowman's glands, atrophy of the nerve bundles, and occasionally deposition of fibrous connective tissue. In some animals, generally those with more severe inflammation, the epithelium replacing the olfactory epithelium was composed of one or multiple layers of flattened squamoid epithelium that was most consistent with recently damaged/lost epithelium undergoing reepithelialization. Necrosis of the olfactory epithelium was characterized by the presence of a segment or cluster of epithelial cells that were shrunken and hypereosinophilic with karyorrhexis and/or nuclear pyknosis with or without cell loss.

Table 17. Incidences of Nonneoplastic Lesions of the Nose in Mice in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Male				
Number Examined Microscopically	50	49	50	50
Foreign Body[a]	1	10**	16**	25**
Hyperostosis	0	0	28** (1.5)[b]	46** (1.6)
Inflammation, Suppurative	14 (1.0)	40** (1.3)	49** (2.6)	48** (2.8)
Lumen, Pigmentation	0	3 (1.0)	4 (1.3)	2 (1.5)
Nasopharyngeal Duct, Degeneration	0	0	4 (1.0)	9** (1.3)
Nasopharyngeal Duct, Inflammation	0	1 (1.0)	2 (1.5)	4 (1.3)
Nasopharyngeal Duct, Metaplasia, Squamous	0	0	0	1 (2.0)
Nerve, Atrophy	0	26** (1.5)	49** (2.9)	50** (3.5)
Olfactory Epithelium, Atrophy	4 (1.5)	24** (1.3)	28** (1.6)	3 (1.0)
Olfactory Epithelium, Fibrosis	0	4 (1.3)	37** (1.8)	43** (2.8)
Olfactory Epithelium, Metaplasia, Respiratory	11 (1.1)	45** (1.5)	49** (3.2)	49** (4.0)
Olfactory Epithelium, Necrosis	0	2 (1.0)	1 (2.0)	0
Respiratory Epithelium, Hyperplasia	5 (1.4)	20** (1.4)	10 (1.3)	19** (1.4)
Respiratory Epithelium, Metaplasia, Squamous	0	14** (1.3)	39** (1.6)	46** (2.3)
Respiratory Epithelium, Necrosis	0	7** (1.0)	16** (1.3)	27** (1.7)

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Septum, Perforation	1	0	26**	37**
Turbinate, Atrophy	0	0	41** (1.3)	50** (2.8)
Female				
Number Examined Microscopically	48	48	50	50
Foreign Body	4	8	13*	17**
Hyperostosis	0	0	21** (1.1)	48** (2.1)
Inflammation, Suppurative	4 (1.0)	24** (1.0)	44** (2.8)	47** (2.4)
Lumen, Pigmentation	0	19** (1.0)	3 (1.0)	16** (1.7)
Nasopharyngeal Duct, Degeneration	0	0	2 (1.0)	4 (1.8)
Nasopharyngeal Duct, Inflammation	0	0	1 (2.0)	3 (1.0)
Nerve, Atrophy	0	13** (1.2)	47** (3.4)	48** (3.5)
Olfactory Epithelium, Atrophy	0	18** (1.6)	26** (1.8)	17** (2.2)
Olfactory Epithelium, Fibrosis	0	1 (1.0)	39** (1.8)	43** (2.2)
Olfactory Epithelium, Metaplasia, Respiratory	2 (1.0)	36** (1.7)	49** (3.4)	48** (3.7)
Olfactory Epithelium, Necrosis	0	2 (1.5)	1 (1.0)	0
Respiratory Epithelium, Hyperplasia	1 (1.0)	1 (1.0)	22** (1.1)	15** (1.3)
Respiratory Epithelium, Metaplasia, Squamous	0	8** (1.0)	42** (2.0)	42** (1.9)
Respiratory Epithelium, Necrosis	0	4 (1.5)	28** (1.3)	32** (1.5)
Septum, Perforation	0	0	38**	42**
Turbinate, Atrophy	0	0	40** (1.2)	48** (2.7)

*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.

In the respiratory epithelium, the incidences of necrosis in all dosed groups except 30 mg/kg females, squamous metaplasia in all dosed groups, and hyperplasia in 30 and 300 mg/kg males and 100 and 300 mg/kg females were significantly greater than the vehicle control incidences (Table 17, Table C-3, and Table D-3). In males, the severity of squamous metaplasia and necrosis increased with increasing dose. Necrosis of the respiratory epithelium was characterized by hypereosinophilia and cell swelling, and often there were large sections of affected epithelium sloughing off of an underlying basal epithelial layer. Squamous metaplasia of the respiratory epithelium was characterized by replacement of the ciliated columnar epithelium by flattened squamous epithelium of variable thickness. This change was located most commonly at the tips of the nasoturbinates and along the lateral wall of Level I, and less often Level II. Respiratory epithelium hyperplasia was characterized by increased numbers of cells piled up in multiple layers or forming folds and invaginations into the underlying lamina propria and was most often observed on the tips of nasal turbinates and along the nasal septum and lateral walls of Levels I and II.

Lung: Increased incidences and severities of inflammation occurred in 100 and 300 mg/kg males; occurrences of the lesion were also recorded in the 30 and 100 mg/kg females [males: 1/50 (1.0), 0/50, 6/50 (4.0), 5/50 (3.2); females: 0/50, 1/49 (4.0), 2/50 (3.0), 0/50; Table C-3 and Table D-3]. Inflammation was characterized primarily by massive infiltrates of neutrophils within alveolar spaces and extending into bronchioles and occasional bronchi. The lesions often were focally extensive over large portions of the affected lung lobes. There were small numbers of alveolar macrophages, and frequently there were large numbers of intracellular and extracellular bacteria admixed with the neutrophils.

Bone Marrow: The incidences of hyperplasia were increased in all dosed groups of males and females, and the increases were significant in all but the 30 mg/kg females (Table 18, Table C-3, and Table D-3). Hyperplasia was characterized by an increase in the myeloid cell population.

Mandibular Lymph Node: The incidences of lymphoid hyperplasia and plasma cell infiltration were significantly increased in 100 and 300 mg/kg males and females (Table 18, Table C-3, and Table D-3). Plasma cell infiltration was characterized by increased plasma cells within the medullary cords. Lymphoid hyperplasia was characterized by expanded follicles and lymphocyte proliferation in paracortical regions.

Liver: The incidences of hepatocellular adenoma (males: vehicle control, 35/50; 30 mg/kg, 29/50; 100 mg/kg, 25/50; 300 mg/kg, 16/50; females: 12/50, 9/50, 3/50, 0/50) in 100 and 300 mg/kg males and females and hepatocellular adenoma or carcinoma (combined) (males: 40/50, 39/50, 35/50, 21/50; females: 14/50, 15/50, 7/50, 1/50) in 300 mg/kg males and 100 and 300 mg/kg females were significantly less than those in the vehicle control groups (Table C-1, Table C-2, Table D-1, and Table D-2). The incidences of multiple hepatocellular adenoma were also significantly decreased in 100 and 300 mg/kg mice (males: 16/50, 15/50, 6/50, 2/50; females: 5/50, 1/50, 0/50, 0/50). Three 30 mg/kg males had hepatocholangiocarcinoma; this incidence was within the NTP historical control range for all routes of administration (range 0%–8%).

The incidences of clear cell foci were significantly decreased in 100 and 300 mg/kg males (24/50, 17/50, 6/50, 1/50) and the incidences of eosinophilic foci were significantly decreased in 300 mg/kg males and 100 and 300 mg/kg females (males: 24/50, 27/50, 22/50, 13/50; females: 23/50, 14/50, 8/50, 3/50; Table C-3 and Table D-3). The incidences of hematopoietic cell proliferation [vehicle control, 2/50 (1.0); 30 mg/kg, 2/50 (1.5); 100 mg/kg, 6/50 (1.2); 300 mg/kg, 10/50 (1.0)] and inflammation [4/50 (1.0), 1/50 (3.0), 5/50, (2.8), 12/50 (1.2)] were significantly increased in 300 mg/kg males. Hematopoietic cell proliferation was composed of multiple small clusters of hematopoietic precursor cells scattered throughout the hepatic parenchyma. The precursor cells were predominantly of granulocytic origin. The hematopoietic cell proliferation seen in the liver was likely similar to the bone marrow hyperplasia and was a response to the continued inflammatory process occurring in the nasal cavities of the mice. Inflammation consisted of a variably mixed infiltrate of neutrophils and macrophages, with or without lymphocytes or plasma cells and was often associated with one or two individual necrotic hepatocytes.

Other Organs: The incidences of adrenal cortical hypertrophy were decreased in 100 and 300 mg/kg males [10/49 (1.4), 8/50 (1.3), 1/50 (2.0), 2/50 (1.5); Table C-3]. The incidence of pancreatic islet hyperplasia was significantly decreased in 300 mg/kg males [22/50 (1.8), 18/50

(1.5), 19/50 (1.4), 1/50 (1.0); Table C-3). Islet cell hyperplasia was characterized by an increase in the size of the pancreatic islets due to an increased number of normal looking islet cells. Affected islets did not compress the adjacent exocrine pancreatic tissue. In general, multiple islets were affected in each animal. Gastrointestinal tract lesions, similar to those observed in the 2-year study in rats, were not observed in mice.

Table 18. Incidences of Selected Nonneoplastic Lesions in Mice in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Male				
Bone Marrow[a]	50	50	50	50
Hyperplasia[b]	5 (1.2)[c]	42** (1.6)	38** (1.6)	46** (1.5)
Mandibular Lymph Node	50	50	50	50
Hyperplasia, Lymphoid	0	1 (1.0)	31** (2.2)	37** (2.1)
Infiltration Cellular, Plasma Cell	1 (1.0)	1 (1.0)	24** (1.3)	41** (1.6)
Female				
Bone Marrow	50	50	50	50
Hyperplasia	6 (2.2)	11 (1.9)	41** (2.4)	34** (1.9)
Mandibular Lymph Node	50	48	49	48
Hyperplasia, Lymphoid	0	1 (2.0)	8** (1.4)	12** (1.4)
Infiltration Cellular, Plasma Cell	0	0	31** (1.3)	18** (1.3)

**Significantly different ($P \leq 0.01$) from the vehicle control group by the Poly-3 test.
[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.

Genetic Toxicology

Green tea extract (50 to 2,000 µg/plate), from the same lot that was tested in the 3-month and 2-year studies, was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 in the presence of induced rat liver S9; no mutagenicity was observed in these strains without S9, or in the *Escherichia coli* strain WP2 *uvrA/*pKM101 (10 to 1,000 µg/plate), with or without S9 (Table E-1).

In vivo, no increases in the frequencies of micronucleated normochromatic erythrocytes were seen in peripheral blood of male or female B6C3F1/N mice administered green tea extract (62.5 to 1,000 mg/kg per day) for 3 months by gavage; no significant changes were observed in the percentage of polychromatic erythrocytes among total erythrocytes in the blood of either males or females, suggesting that green tea extract did not induce bone marrow toxicity (Table E-2).

Figure 10. Macroscopic Aspect of the Mucosa in the Duodenum of a Male Wistar Han Rat Administered 1,000 mg/kg Green Tea Extract by Gavage for Two Years

Note the black discoloration.

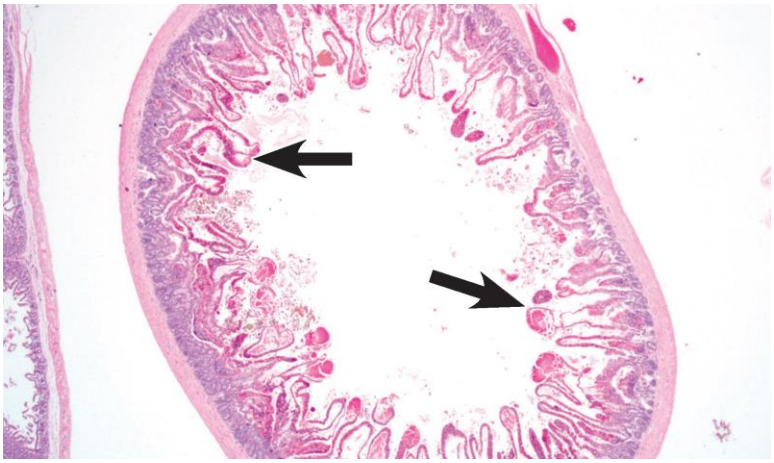

Figure 11. Necrosis of the Mucosa in the Duodenum of a Male Wistar Han Rat Administered 1,000 mg/kg Green Tea Extract by Gavage for Two Years (H&E)

Note the hypereosinophilic aspect (arrows) of the epithelial cells located at the tips of villi or for variable lengths along the villi. The hypereosinophilic aspect is due to pyknotic necrosis of the cells associated with the presence of fibrin thrombi within the lamina propria.

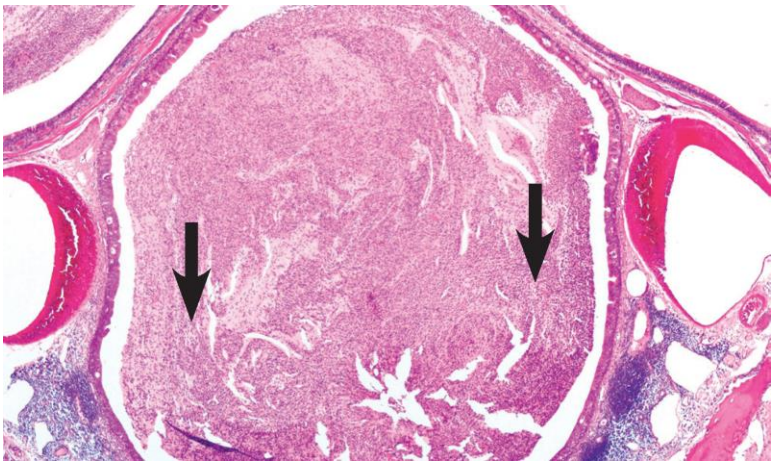

Figure 12. Suppurative Inflammation (Arrows) in the Nasopharyngeal Duct of a Male Wistar Han Rat Administered 1,000 mg/kg Green Tea Extract by Gavage for Two Years (H&E)

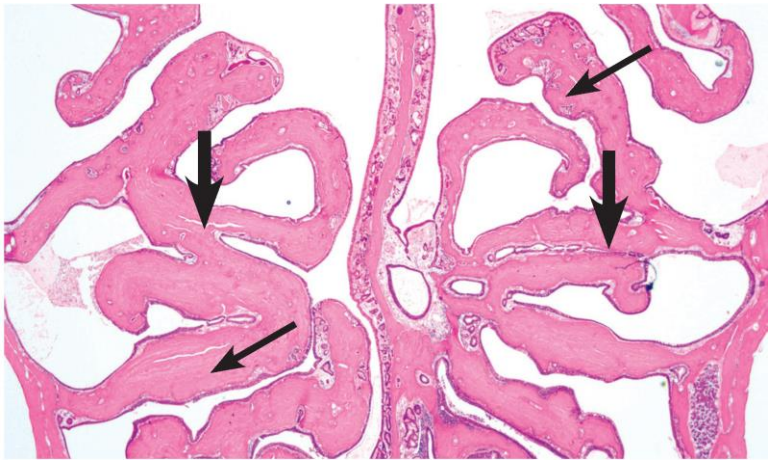

Figure 13. Increased Bony Deposition (Hyperostosis) and Deformity in the Nasal Turbinate of a Male Wistar Han Rat Administered 1,000 mg/kg Green Tea Extract by Gavage for Two Years (H&E)

Note the thickening of the nasal turbinates (small arrows) with occasional fusion of turbinates to each other (large arrows).

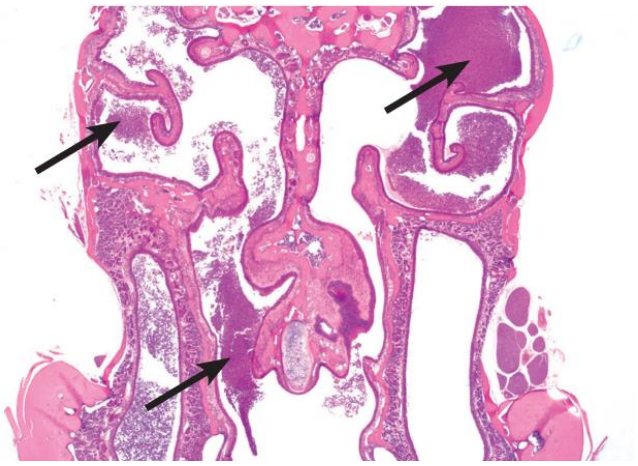

Figure 14. Section of the Nose, Level III, from a Female B6C3F1/N Mouse Administered 300 mg/kg Green Tea Extract by Gavage for Two Years (H&E)

Note the suppurative inflammation within the lumen (arrows). The inflammation also involved the underlying mucosa.

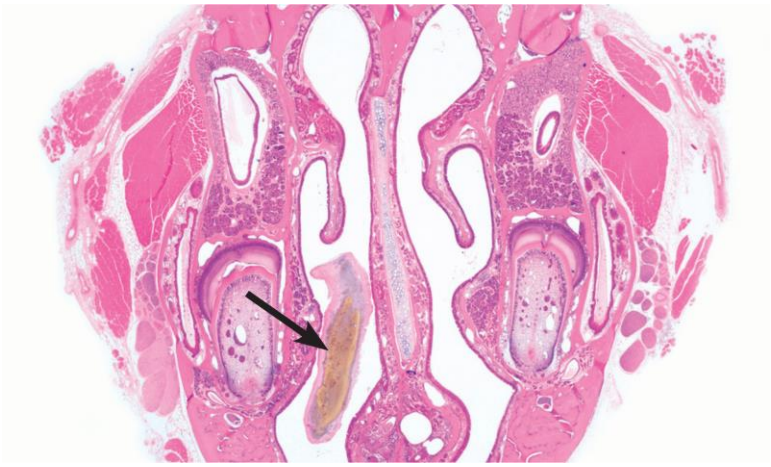

Figure 15. Section of the Nose, Level II, from a Female B6C3F1/N Mouse Administered 300 mg/kg Green Tea Extract by Gavage for Two Years (H&E)

Note the greenish brown material within the lumen (arrow), thought to be the test compound.

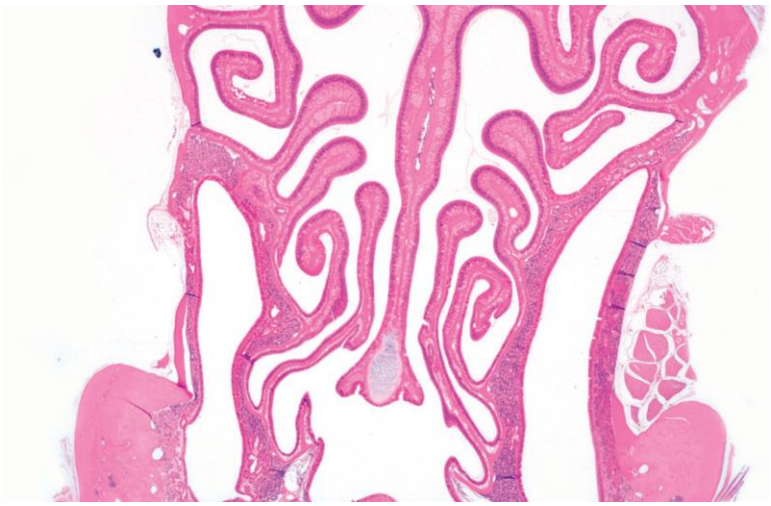

Figure 16. Section of the Nose, Level III, from a Vehicle Control Male B6C3F1/N Mouse in the Two-ear Gavage Study of Green Tea Extract (H&E)

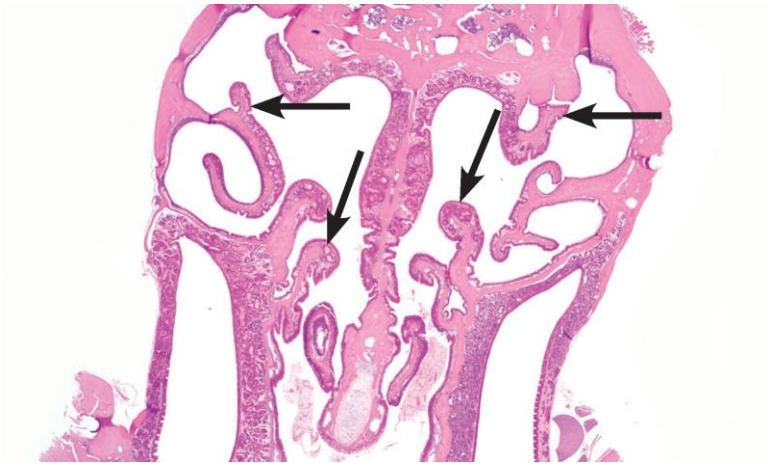

Figure 17. Section of the Nose, Level III, from a Male B6C3F1/N Mouse Administered 100 mg/kg Green Tea Extract by Gavage for Two Years (H&E)

Note the blunted atrophic turbinates (arrows) compared to the normal turbinates in Figure 16.

Discussion

Green tea extract is the purported active ingredient of many weight loss and nutritional supplements. Also, green tea extract, or components thereof, are currently being investigated in multiple human clinical trials as a potential treatment for various cancers, hypercholesterolemia, diabetes, and cardiovascular diseases. Despite widespread use, the potential adverse effects of repeated chronic consumption of green tea extract supplements, which contain constituent levels higher than the traditional green tea beverage, have not been adequately investigated. Hence, green tea extract was tested by NTP to characterize its potential subchronic and chronic toxicity and carcinogenicity. NTP conducted 3-month toxicology studies of green tea extract in F344/NTac rats and B6C3F1/N mice and 2-year toxicology and carcinogenicity studies in Wistar Han rats and B6C3F1/N mice. A gavage route of administration was selected to mimic human consumption of supplements. A 3-month interim evaluation was conducted during the 2-year rat study to compare high dose effects (1,000 mg/kg) between F344/NTac and Wistar Han rats.

Prior to the toxicity evaluation, green tea extract lots were characterized by NTP. A number of green tea extract formulations with varying concentrations of active ingredient are available as over-the-counter products in grocery stores and pharmacies in the United States. Comparative studies have been performed on a few of the commercially available products to determine the catechin polyphenol and caffeine content of different preparations[6; 96-98] and results demonstrated a wide variation in concentrations of catechins and caffeine in different green tea extract products. In addition, the measured catechin polyphenol and caffeine content in many products did not match their corresponding label claims. Caffeine was found to be present in most of the products but only a few products made label claims about the quantities of caffeine present[6; 96]. For the current studies, NTP conducted a chemical content analysis on four commercially available products and selected a representative green tea extract. Selection was based upon concentrations of EGCG, lack of adulteration, similarity with other products on the market, and availability in bulk quantity. The proportion of catechin polyphenols in NTP's test article was similar to that in Standard Reference Material 3255 identified by the National Institute of Standards and Technology[95].

Doses for the 3-month studies in rats and mice were selected based on a 3-month study conducted by the National Cancer Institute (NCI) using 1,000 mg/kg of green tea catechin polyphenols as the highest dose in Harlan Sprague-Dawley rats[136]. The abstract for that study described increased treatment-related deaths in the 1,000 mg/kg group, dose-related suppression of body weight gains, and reduced spleen and thymus weights. Intestinal dilation was noted in many of the early death and terminally killed animals. NTP selected a top dose of 1,000 mg/kg for the current 3-month studies because it was not known if F344/NTac rats and B6C3F1/N mice were more or less sensitive to green tea extract administration. Additionally, because the majority of botanical extracts are not standardized, there was a high probability that substantial variability in concentrations of catechin polyphenols would exist between the two test green tea extracts used by the NCI and NTP.

In the current 3-month F344/NTac rat study, there were no treatment-related adverse effects on survival. However, administration of 250 mg/kg or greater was associated with significant decreases in body weights (5% to 14%) in male and female rats. Histologic lesions associated with green tea extract administration occurred in the liver (females only), nose, lymph nodes

(males only), and thymus of rats and the incidences of these lesions increased in a dose-dependent manner. Because administration of 1,000 mg/kg had no effect on survival and the reductions in body weights and the histopathologic lesions were not considered to be dose limiting, a high dose of 1,000 mg/kg was selected for the 2-year studies in Wistar Han rats. Consistently, Wistar Han rats administered 1,000 mg/kg and evaluated at 3 months displayed little or no reductions in body weight. Mice appeared to be more sensitive in the 3-month study with regard to effects on survival following green tea extract administration, because significant mortality was observed in males and females administered 1,000 mg/kg. The treatment-related decreases in final mean body weights of males and females administered 250 mg/kg or greater ranged from 12% to 24%. Treatment-related lesions were observed in the liver, nose, lymph nodes, spleen (females only), and thymus in mice. In male mice, significant increases in the incidences of various nonneoplastic lesions of the nose were observed in the 250 mg/kg or greater groups. Based on these observations, 300 mg/kg was selected as the high dose for 2-year studies in B6C3F1/N mice.

In the 2-year Wistar Han rat study, survival of 1,000 mg/kg males and females was significantly less than survival of the vehicle control groups. No signs of overt toxicity were observed in the rats except decreased body weights in male rats. The increase in mortality in 1,000 mg/kg female rats did not correlate with decreases in body weight. The increased deaths in green tea extract treated rats could be ascribed to gastrointestinal toxicity. Macroscopic analysis of the gastrointestinal tract showed dose-related increased incidences of gray to black, focal to diffuse discoloration of the mucosa and wall of the stomach and small intestine. Previous studies of green tea extract toxicity in Harlan Sprague-Dawley rats (fed) and beagle dogs (fasted) demonstrated similar gross histopathologic changes in the gastrointestinal tract and unexpected mortality[33; 136]. The increased mortality in rats could also partly be related to dose-dependent increases in nasal lesions in male and female rats. At 2 years, a spectrum of inflammatory and degenerative lesions in the nose was observed in all dosed groups of rats.

Decreased body weight gain after green tea extract consumption has been previously demonstrated in both animals and humans[58; 137-139]. The mechanisms of dose-dependent decreases in body weight in the 3-month and 2-year rat and mouse studies are not known, and the decreases might be attributed to decreased feed consumption and inhibition of intestinal nutrient absorption[57; 140; 141]. Green tea extract is rich in catechin polyphenols, which are also classified as condensed tannins. Studies in experimental animals have associated consumption of feed rich in condensed tannins to decreased feed intake, protein digestibility, and growth rate[142]. Condensed tannins have been shown to inhibit numerous digestive enzymes such as cellulose, pectinase, amylase, lipase, proteolytic enzymes, and β-galactosidase in vitro. It has also been reported that dietary tannins form complexes with dietary protein and reduce the digestibility and absorption of proteins[142; 143].

In the current 3-month and 2-year studies, numerous histopathologic lesions occurred in the nose of F344/NTac rats, Wistar Han rats, and B6C3F1/N mice. In the 3-month studies, green tea extract administration significantly increased the incidences of inflammation (female F344/NTac rats only), hyperplasia of the Bowman's gland underlying the olfactory epithelium (male and female F344/NTac rats only), nerve atrophy and atrophy of the olfactory epithelium, metaplasia and pigmentation (male and female F344/NTac rats only) of the olfactory epithelium, and necrosis of the olfactory epithelium (female B6C3F1/N mice only). Similar histopathologic

changes of the nose were also observed in male and female Wistar Han rats at 3 months, but to a lesser extent than in F344/NTac rats.

In the 2-year studies in Wistar Han rats and B6C3F1/N mice, the most pronounced response was observed in the nose. Treatment with green tea extract produced a spectrum of inflammatory, degenerative, and proliferative lesions of the olfactory epithelium, nasopharyngeal duct, and respiratory epithelium in rats and mice. Significant and progressive increases in the incidences of inflammation, atrophy of the olfactory epithelium, nerve atrophy, metaplasia of the olfactory epithelium, and necrosis (rats only) occurred in both rats and mice. A treatment-related increase in the incidences of suppurative inflammation of the nasopharyngeal duct was noted in male and female rats. In addition, dose-dependent increases in excessive growth of turbinate bone (hyperostosis) and deformity were observed in Level III of the nose of dosed groups of rats while turbinate atrophy was observed in most 100 and 300 mg/kg male and female mice. Another distinct finding was the significantly increased occurrences of foreign body in the nose of male and female mice and female rats in the 2-year studies. In the 2-year mouse study, the nasal lesions were observed even in the low dose group (30 mg/kg). Inflammatory responses were also observed in the lung and epicardium of the heart in male and female rats during the 2-year study.

The nasal toxicity observed following oral exposure to green tea extract may be related to direct systemic toxicity of green tea extract or its metabolites. A significant amount of metabolizing enzyme activity is known to occur in the nasal epithelia in many species[144; 145]. Some of the active metabolizing enzymes present in nasal epithelium include CYP 450, glutathione-S-transferase, carboxylesterase, aldehyde dehydrogenase and flavin monooxygenases[146]. Green tea catechins are known to undergo rapid and extensive metabolic transformations. Therefore, one of the potential mechanisms underlying the unexpected nasal toxicity observed may be the metabolic activation of green tea extract components. Another potential mechanism of the pathogenesis of nasal toxicity could be the retrograde aspiration of green tea extract or stomach acids into the nasal cavity. Articles published in the literature have described the characteristic histopathologic changes indicative of gavage-related reflux of irritant compounds[147]. In the current 2-year studies, increased lesion incidences were observed in the posterior nose levels (Level II and Level III) and the presence of foreign bodies (which may have been the test article or feed material) were evident in a significant number of rats and mice in the highest dosed groups. These observations have been described as typical of gavage-related reflux and subsequent aspiration of irritant test material[147; 148]. Lesions observed in the lung and heart may represent an extension of the inflammatory process secondary to aspiration of test material.

Another important target organ system for green tea extract was found to be the gastrointestinal tract in rats. At 2 years, dose-dependent increases in the incidences of gray to black focal to diffuse discoloration were observed macroscopically in the stomach mucosa and small intestine of rats. These gross changes coincided with significant dose-dependent increases in the incidences of mucosa necrosis of the glandular stomach and all segments of the small intestine in males and females. These lesions throughout the gastrointestinal tract were primarily observed in the 1,000 mg/kg groups and correlated with the low survival observed in these groups. Microscopic evaluation of the necrotic tissue showed evidence of congestion and hemorrhage in the stomach and small intestine of a few rats. The green tea extract-induced gastrointestinal toxicity was consistent with previously published studies in animals and humans[33; 63]. In humans, severe rectal bleeding was reported in one female patient with a previous history of gastrointestinal bleeding during a Phase IB clinical trial of green tea extract conducted by NCI[63].

Although the exact causes and mechanisms of observed gastrointestinal injury in the rats are not completely understood, injury could be attributed to administration of high amounts of condensed tannins or catechin polyphenols in the test compound. Previous studies have demonstrated that exposure to high amounts of tannins could cause gastroenteritis and intestinal wall congestion in rats[149] and hemorrhagic gastroenteritis in rabbits[150]. In contrast to the rats, no evidence of gastrointestinal injury was noted in the 2-year study in mice. The lack of gastrointestinal toxicity in mice may be attributed to the relatively lower doses used in the 2-year mouse study compared to the 2-year rat study.

The gavage route of administration may have contributed to observed toxicity (e.g., gastrointestinal toxicity and effects on survival) in rats. When a catechin mixture was administered in feed (0.02%, 0.3%, 1%, or 3%) in a 2-year exposure scenario, only effects on body weight and relative liver weights with centrilobular hypertrophy were noted[60]. The high dose of 3% in the feed resulted in doses of 1,265.8 and 1,539.8 mg/kg per day (respectively) in male and female rats, higher than the 1,000 mg/kg dose used in the current study, suggesting that the co-administration of green tea catechins with food reduces the potential for chronic toxicity.

Case reports describe occasional cases of hepatotoxicity and liver failure following green tea extract use in humans[7; 61; 63; 151; 152]. The potential hepatotoxicity leading to liver failure is a major safety concern in using green tea extract products[7]. At 3 months in the NTP studies, histopathologic changes were noted in the liver of female F344/NTac and Wistar Han rats and in male and female B6C3F1/N mice in the 1,000 mg/kg groups. In female F344/NTac and Wistar Han rats at3 months, the liver lesions included inflammation, minimal bile duct hyperplasia, oval cell hyperplasia, hepatocyte hypertrophy, periportal hypertrophy, minimal to mild mitosis, and/or hepatocyte necrosis. Supportive evidence from clinical chemistry analyses showed significant increases in bile salt concentrations and alanine aminotransferase activities in the serum of the same F344/NTac rats at 3 months. Clinical chemistry endpoints were not measured in the Wistar Han rats at 3 months. Based on the incidences of hepatic necrosis, the mice showed relatively greater levels of hepatic toxicity than the rats following 3 months of dosing. Similarly, reports in the literature have found mice to be more sensitive to green tea extract-induced hepatic toxicity[58]. These differences could be related to the greater bioavailability of EGCG in mice (26%) than in rats (1.6%)[21; 22].

In the 2-year Wistar Han rat study, the incidences of hepatic necrosis were significantly increased only in the 1,000 mg/kg males and females, where decreases in survival were observed. Increased incidences of inflammation and hematopoietic cell proliferation were noted in 300 mg/kg male mice in the 2-year study. No pathological changes in the liver of female mice were related to green tea administration in the 2-year study. No obvious species differences in toxicity were noted in the 2-year studies.

There were gender differences in the sensitivity to induced hepatic toxicity in F344/NTac and Wistar Han rats exposed to green tea extract in the current studies. At 3 months, only a few female F344/NTac rats in the 1,000 mg/kg group developed hepatic necrosis and inflammation. At 2 years, although both male and female Wistar Han rats had increased incidences of hepatic necrosis, the incidences were higher in females (52%) than in males (26%) in the 1,000 mg/kg group. These findings are corroborated by reports in the literature that females appear to be more susceptible to EGCG and green tea extract-induced hepatotoxicity in animals as well as humans[61; 153].

In the 3-month and 2-year studies, effects of green tea extract administration were also observed in lymphoid organs such as the lymph nodes, spleen, and thymus. Significant decreases in spleen weights (male rats) and thymus weights (male and female rats and mice) were observed in the 3-month studies. Corresponding increased incidences of thymus atrophy were observed microscopically in male and female rats and mice in the 1,000 mg/kg groups. Other histopathologic changes in lymphoid organs in mice administered green tea extract for 3 months included atrophy of the mandibular lymph node (male and female mice) and lymphoid atrophy of the spleen (female mice) in the 1,000 mg/kg groups. In addition, significant dose-related increases in the incidences of bone marrow hyperplasia were observed in female rats and male and female mice in the 2-year study. These changes may have been in response to the effects that occurred in non-lymphoid organs, e.g., nose, and thus secondary alterations in response to green tea administration.

The NTP in vivo micronucleus test results with green tea extract were negative in mice and are similar to observations from several other in vivo genotoxicity tests of green tea extract or EGCG under a variety of exposure conditions (e.g., dietary, gavage, injection, single versus multiple exposures) and with different preparations of green tea extracts[80; 81; 85; 87]. The lack of genotoxic effects from exposure to green tea extract in vivo is consistent with the overall lack of carcinogenic activity in the 2-year bioassay.

In summary, the current 2-year studies of green tea extract identified liver, nose, and gastrointestinal tract (rats only) as the major targets for toxicity in Wistar Han rats and B6C3F1/N mice. This is the first report describing green tea extract-induced nasal lesions in rats and mice. Nasal toxicity associated with the administration of green tea extract for 2 years occurred in all dosed groups of rats and mice, even in the 30 mg/kg groups of mice. The observed nasal toxicity could be related to systemic exposure to green tea extract or its metabolites. However, the pattern of nasal changes observed suggests that gavage-related reflux of green tea extract or stomach contents could also be a potential inducer of nasal toxicity in rats and mice. At 2 years, significant necrosis was also noted in the gastrointestinal tract of 1,000 mg/kg male and female rats. Increased incidences of hepatic necrosis were noted in the 3-month studies of F344/N/Tac rats and B6C3F1/N mice and in the 2-year study in Wistar Han rats. Female rats appeared to be more susceptible to the liver toxicity of green tea extract in the 3-month and 2-year studies. The findings of liver and gastrointestinal toxicities correlate with reported incidences of hepatic necrosis and rectal bleeding in humans. The current studies indicate that green tea extract has the potential to cause hepatic and gastrointestinal toxicity when administered for prolonged periods in rats and mice.

Conclusions

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity*[a] of green tea extract in male or female Wistar Han rats administered 100, 300, or 1,000 mg/kg. There was *no evidence of carcinogenic activity* of green tea extract in male or female B6C3F1/N mice administered 30, 100, or 300 mg/kg.

Administration of green tea extract resulted in increased incidences of nonneoplastic lesions of the liver, glandular stomach, small intestine (duodenum, ileum, and jejunum), nose, lung, heart, and spleen in male and female rats; bone marrow of female rats; the nose, mandibular lymph node, and bone marrow of male and female mice; and the liver of male mice.

[a]See Explanation of Levels of Evidence of Carcinogenic Activity. See summary of the peer review panel comments and the public discussion on this Technical Report in Appendix L.

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Appendix A. Summary of Lesions in Male Wistar Han Rats in the Two-year Gavage Study of Green Tea Extract

Tables

Table A-1. Summary of the Incidence of Neoplasms in Male Rats in the Two-year Gavage Study of Green Tea Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
Three-month interim evaluation	10	–	–	10
Early deaths				
Accidental deaths	–	–	–	2
Moribund	12	7	5	5
Natural deaths	3	6	2	19
Survivors				
Died last week of study	–	–	1	–
Terminal kill	35	37	42	24
Animals examined microscopically	60	50	50	60
Systems Examined at Three Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
Two-year Study				
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Leiomyosarcoma, metastatic, stomach, forestomach	1 (2%)	–	–	–
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)	(47)	(49)	(48)
Intestine small, ileum	(50)	(48)	(49)	(45)
Intestine small, jejunum	(49)	(47)	(48)	(46)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	–	–	–	1 (2%)
Mesentery	(3)	(2)	(1)	(1)
Schwannoma malignant	1 (33%)	–	1 (100%)	–
Oral mucosa	(1)	(0)	(0)	(0)
Gingival, squamous cell carcinoma	1 (100%)	–	–	–
Pancreas	(50)	(50)	(50)	(50)
Carcinoma, metastatic, prostate	–	1 (2%)	–	–
Leiomyosarcoma, metastatic, stomach, forestomach	1 (2%)	–	–	–
Salivary glands	(50)	(50)	(50)	(48)
Stomach, forestomach	(50)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)	–	–	–
Stomach, glandular	(49)	(50)	(50)	(50)
Tooth	(1)	(1)	(0)	(0)
Odontoma	–	1 (100%)	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Adventitia, aorta, leiomyosarcoma, metastatic, stomach, forestomach	1 (2%)	–	–	–
Heart	(50)	(50)	(50)	(50)
Leiomyosarcoma, metastatic, stomach, forestomach	1 (2%)	–	–	–
Schwannoma malignant	–	1 (2%)	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	–	–	2 (4%)
Osteosarcoma, metastatic, bone	1 (2%)	–	–	–
Adrenal medulla	(50)	(50)	(50)	(50)
Osteosarcoma, metastatic, bone	1 (2%)	–	–	–
Pheochromocytoma benign	1 (2%)	1 (2%)	1 (2%)	–
Pheochromocytoma complex	–	1 (2%)	–	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	1 (2%)
Parathyroid gland	(47)	(44)	(49)	(45)
Adenoma	–	–	–	1 (2%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	16 (32%)	15 (30%)	14 (28%)	10 (20%)
Pars distalis, adenoma, multiple	1 (2%)	2 (4%)	3 (6%)	–
Pars distalis, carcinoma	–	–	2 (4%)	–
Pars intermedia, adenoma	1 (2%)	2 (4%)	–	1 (2%)
Pars nervosa, adenoma	–	–	1 (2%)	–
Thyroid gland	(50)	(50)	(50)	(49)
Bilateral, C-cell, adenoma	–	1 (2%)	1 (2%)	–
C-cell, adenoma	4 (8%)	3 (6%)	5 (10%)	5 (10%)
C-cell, carcinoma	–	–	1 (2%)	–
Follicular cell, adenoma	–	3 (6%)	1 (2%)	2 (4%)
Follicular cell, adenoma, multiple	–	–	1 (2%)	–
General Body System				
None	–	–	–	–
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)	–	–	–
Prostate	(50)	(50)	(50)	(50)
Carcinoma	–	1 (2%)	–	–
Seminal vesicle	(50)	(50)	(50)	(50)
Carcinoma, metastatic, prostate	–	1 (2%)	–	–
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	2 (4%)	1 (2%)	1 (2%)	–
Interstitial cell, adenoma, multiple	–	1 (2%)	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(4)	(0)	(1)	(1)
Lymph node, mandibular	(49)	(50)	(50)	(48)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemangioma	–	–	1 (2%)	–
Hemangiosarcoma	–	1 (2%)	–	–
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, Lymph node, mesenteric	–	1 (2%)	–	–

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Thymus	(49)	(49)	(50)	(50)
Thymoma benign	2 (4%)	1 (2%)	3 (6%)	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibroadenoma	–	1 (2%)	–	–
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	2 (4%)	3 (6%)	–	–
Basosquamous tumor malignant	–	1 (2%)	–	–
Hamartoma	1 (2%)	–	–	–
Keratoacanthoma	2 (4%)	–	2 (4%)	–
Squamous cell carcinoma	1 (2%)	–	–	–
Squamous cell papilloma	1 (2%)	–	3 (6%)	–
Trichoepithelioma	–	1 (2%)	1 (2%)	–
Sebaceous gland, adenoma	1 (2%)	–	–	–
Subcutaneous tissue, fibroma	1 (2%)	2 (4%)	–	–
Subcutaneous tissue, lipoma	1 (2%)	–	1 (2%)	–
Subcutaneous tissue, sarcoma, metastatic, bone	1 (2%)	–	–	–
Subcutaneous tissue, schwannoma malignant	2 (4%)	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteoma	1 (2%)	–	–	–
Osteosarcoma	1 (2%)	–	–	–
Joint, sarcoma	1 (2%)	–	–	–
Skeletal muscle	(0)	(0)	(0)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma malignant	1 (2%)	–	2 (4%)	1 (2%)
Granular cell tumor benign	2 (4%)	2 (4%)	1 (2%)	–
Meningioma benign	–	–	1 (2%)	–
Squamous cell carcinoma, metastatic, oral mucosa	1 (2%)	–	–	–
Peripheral nerve	(0)	(1)	(0)	(1)
Spinal cord	(0)	(1)	(0)	(1)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	–	–	1 (2%)	–
Carcinoma, metastatic, prostate	–	1 (2%)	–	–
Carcinoma, metastatic, thyroid gland	–	–	1 (2%)	–
Leiomyosarcoma, metastatic, stomach, forestomach	1 (2%)	–	–	–
Osteosarcoma, metastatic, bone	1 (2%)	–	–	–
Schwannoma, malignant, metastatic, skin	1 (2%)	–	–	–
Squamous cell carcinoma, metastatic, skin	1 (2%)	–	–	–
Nose	(50)	(50)	(50)	(50)
Adenoma	–	–	1 (2%)	–
Squamous cell carcinoma, metastatic, oral mucosa	1 (2%)	–	–	–
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, oral mucosa	1 (2%)	–	–	–
Zymbal's gland	(1)	(0)	(0)	(0)
Carcinoma	1 (100%)	–	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Lipoma	–	–	1 (2%)	–
Liposarcoma	1 (2%)	–	–	–
Osteosarcoma, metastatic, bone	1 (2%)	–	–	–
Renal tubule, carcinoma	–	1 (2%)	–	–
Ureter	(1)	(0)	(0)	(0)
Liposarcoma, metastatic, kidney	1 (100%)	–	–	–
Urinary bladder	(50)	(50)	(50)	(50)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Lymphoma malignant	–	–	1 (2%)	–
Mesothelioma malignant	2 (4%)	–	–	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]				
Two-year study	36	34	32	18
Total primary neoplasms				
Two-year study	54	47	52	26
Total animals with benign neoplasms				
Two-year study	26	31	30	17
Total benign neoplasms				
Two-year study	40	41	44	23
Total animals with malignant neoplasms				
Two-year study	12	6	6	2
Total malignant neoplasms				
Two-year study	14	6	8	3
Total animals with metastatic neoplasms				
Two-year study	6	2	1	–
Total metastatic neoplasms				
Two-year study	16	4	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 A-2. Statistical Analysis of Primary Neoplasms in Male Rats in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate[a]	17/50 (34%)	17/50 (34%)	17/50 (34%)	10/50 (20%)
Adjusted rate[b]	37.4%	35.8%	35.5%	27.9%
Terminal rate[c]	12/35 (34%)	9/37 (24%)	15/43 (35%)	5/24 (21%)
First incidence (days)	505	541	585	554
Poly-3 test[d]	P = 0.231N	P = 0.524N	P = 0.512N	P = 0.256N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	17/50 (34%)	17/50 (34%)	19/50 (38%)	10/50 (20%)
Adjusted rate	37.4%	35.8%	39.6%	27.9%
Terminal rate	12/35 (34%)	9/37 (24%)	16/43 (37%)	5/24 (21%)
First incidence (days)	505	541	585	554
Poly-3 test	P = 0.239N	P = 0.524N	P = 0.496	P = 0.256N
Skin: Squamous Cell Papilloma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.3%	0.0%	6.4%	0.0%
Terminal rate	1/35 (3%)	0/37 (0%)	3/43 (7%)	0/24 (0%)
First incidence (days)	727 (T)	–[e]	727 (T)	–
Poly-3 test	P = 0.541N	P = 0.493N	P = 0.334	P = 0.549N
Skin: Basal Cell Adenoma				
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	4.6%	6.6%	0.0%	0.0%
Terminal rate	2/35 (6%)	2/37 (5%)	0/43 (0%)	0/24 (0%)
First incidence (days)	727 (T)	636	–	–
Poly-3 test	P = 0.123N	P = 0.518	P = 0.220N	P = 0.296N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	3/50 (6%)	0/50 (0%)	5/50 (10%)	0/50 (0%)
Adjusted rate	6.9%	0.0%	10.6%	0.0%
Terminal rate	3/35 (9%)	0/37 (0%)	5/43 (12%)	0/24 (0%)
First incidence (days)	727 (T)	–	727 (T)	–
Poly-3 test	P = 0.312N	P = 0.113N	P = 0.400	P = 0.170N
Skin: Trichoepithelioma or Basal Cell Adenoma				
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.6%	8.7%	2.1%	0.0%
Terminal rate	2/35 (6%)	2/37 (5%)	1/43 (2%)	0/24 (0%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
First incidence (days)	727 (T)	513	727 (T)	–
Poly-3 test	P = 0.121N	P = 0.363	P = 0.473N	P = 0.296N
Skin: Trichoepithelioma, Basal Cell Adenoma, or Malignant Basosquamous Tumor				
Overall rate	2/50 (4%)	5/50 (10%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.6%	10.9%	2.1%	0.0%
Terminal rate	2/35 (6%)	3/37 (8%)	1/43 (2%)	0/24 (0%)
First incidence (days)	727 (T)	513	727 (T)	–
Poly-3 test	P = 0.095N	P = 0.239	P = 0.473N	P = 0.296N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	4/50 (8%)	0/50 (0%)	5/50 (10%)	0/50 (0%)
Adjusted rate	9.1%	0.0%	10.6%	0.0%
Terminal rate	3/35 (9%)	0/37 (0%)	5/43 (12%)	0/24 (0%)
First incidence (days)	519	–	727 (T)	–
Poly-3 test	P = 0.226N	P = 0.058N	P = 0.539	P = 0.102N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, Malignant Basosquamous Tumor, or Squamous Cell Carcinoma				
Overall rate	6/50 (12%)	5/50 (10%)	6/50 (12%)	0/50 (0%)
Adjusted rate	13.6%	10.9%	12.8%	0.0%
Terminal rate	5/35 (14%)	3/37 (8%)	6/43 (14%)	0/24 (0%)
First incidence (days)	519	513	727 (T)	–
Poly-3 test	P = 0.048N	P = 0.472N	P = 0.576N	P = 0.035N
Thymus: Benign Thymoma				
Overall rate	2/49 (4%)	1/49 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.7%	2.3%	6.4%	0.0%
Terminal rate	2/34 (6%)	0/36 (0%)	2/43 (5%)	0/24 (0%)
First incidence (days)	727 (T)	661	646	–
Poly-3 test	P = 0.303N	P = 0.486N	P = 0.549	P = 0.291N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	4/50 (8%)	4/50 (8%)	6/50 (12%)	5/49 (10%)
Adjusted rate	9.1%	8.8%	12.8%	14.6%
Terminal rate	3/35 (9%)	3/37 (8%)	6/43 (14%)	3/24 (13%)
First incidence (days)	505	513	727 (T)	611
Poly-3 test	P = 0.261	P = 0.626N	P = 0.409	P = 0.346
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	7/50 (14%)	5/49 (10%)
Adjusted rate	9.1%	8.8%	14.9%	14.6%

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Terminal rate	3/35 (9%)	3/37 (8%)	6/43 (14%)	3/24 (13%)
First incidence (days)	505	513	701	611
Poly-3 test	P = 0.260	P = 0.626N	P = 0.299	P = 0.346
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	2/49 (4%)
Adjusted rate	0.0%	6.7%	4.3%	5.9%
Terminal rate	0/35 (0%)	3/37 (8%)	2/43 (5%)	2/24 (8%)
First incidence (days)	–	727 (T)	727 (T)	727 (T)
Poly-3 test	P = 0.336	P = 0.124	P = 0.255	P = 0.186
All Organs: Benign Neoplasms				
Overall rate	26/50 (52%)	31/50 (62%)	30/50 (60%)	17/50 (34%)
Adjusted rate	56.7%	63.4%	61.1%	46.0%
Terminal rate	20/35 (57%)	20/37 (54%)	26/43 (61%)	9/24 (38%)
First incidence (days)	505	513	224	525
Poly-3 test	P = 0.119N	P = 0.322	P = 0.411	P = 0.226N
All Organs: Malignant Neoplasms				
Overall rate	12/50 (24%)	6/50 (12%)	6/50 (12%)	2/50 (4%)
Adjusted rate	25.2%	13.1%	12.5%	5.8%
Terminal rate	3/35 (9%)	4/37 (11%)	4/43 (9%)	1/24 (4%)
First incidence (days)	519	513	224	611
Poly-3 test	P = 0.034N	P = 0.110N	P = 0.091N	P = 0.023N
All Organs: Benign or Malignant Neoplasms				
Overall rate	36/50 (72%)	34/50 (68%)	32/50 (64%)	18/50 (36%)
Adjusted rate	72.0%	69.2%	65.0%	48.7%
Terminal rate	21/35 (60%)	22/37 (60%)	27/43 (63%)	10/24 (42%)
First incidence (days)	505	513	224	525
Poly-3 test	P = 0.016N	P = 0.468N	P = 0.296N	P = 0.022N

T = terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for pituitary gland, thymus, 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 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 A-3. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Two-year Gavage Study of Green Tea Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
Three-month interim evaluation	10	–	–	10
Early deaths				
Accidental deaths	–	–	–	2
Moribund	12	7	5	5
Natural deaths	3	6	2	19
Survivors				
Died last week of study	–	–	1	–
Terminal kill	35	37	42	24
Animals examined microscopically	60	50	50	60
Three-month interim evaluation				
Alimentary System				
Intestine large, cecum	(10)	(0)	(0)	(10)
Epithelium, hyperplasia	–	–	–	1 (10%)
Liver	(10)	(0)	(0)	(10)
Hematopoietic cell proliferation	1 (10%)	–	–	–
Cardiovascular System				
Heart	(10)	(0)	(0)	(10)
Cardiomyopathy	2 (20%)	–	–	–
Genital System				
Preputial gland	(10)	(0)	(0)	(10)
Inflammation	1 (10%)	–	–	1 (10%)
Prostate	(10)	(0)	(0)	(10)
Inflammation	2 (20%)	–	–	–
Hematopoietic System				
Lymph node, mandibular	(10)	(0)	(0)	(10)
Hyperplasia, plasma cell	5 (50%)	–	–	6 (60%)
Respiratory System				
Lung	(10)	(0)	(0)	(10)
Inflammation, chronic active	1 (10%)	–	–	3 (30%)
Alveolus, infiltration cellular, histiocyte	2 (20%)	–	–	2 (20%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Nose	(10)	(0)	(0)	(10)
Foreign body	–	–	–	1 (10%)
Inflammation, suppurative	–	–	–	2 (20%)
Inflammation, acute	–	–	–	1 (10%)
Epithelium, nasopharyngeal duct, hyperplasia	–	–	–	1 (10%)
Epithelium, nasopharyngeal duct, necrosis	–	–	–	1 (10%)
Epithelium, nasopharyngeal duct, pigmentation	–	–	–	1 (10%)
Goblet cell, hyperplasia	1 (10%)	–	–	2 (20%)
Lamina propria, mineralization	–	–	–	1 (10%)
Nasopharyngeal duct, foreign body	–	–	–	1 (10%)
Nasopharyngeal duct, inflammation, suppurative	–	–	–	2 (20%)
Nerve, atrophy	–	–	–	5 (50%)
Olfactory epithelium, atrophy	–	–	–	5 (50%)
Olfactory epithelium, metaplasia, respiratory	–	–	–	3 (30%)
Olfactory epithelium, necrosis	–	–	–	2 (20%)
Olfactory epithelium, pigmentation	–	–	–	3 (30%)
Respiratory epithelium, hyperplasia	2 (20%)	–	–	3 (30%)
Respiratory epithelium, metaplasia, squamous	–	–	–	2 (20%)
Urinary System				
Kidney	(10)	(0)	(0)	(10)
Inflammation	1 (10%)	–	–	–
Nephropathy	–	–	–	1 (10%)
Systems Examined at Three Months with No Lesions Observed				
Endocrine System				
General Body System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Two-year Study				
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)	–	–
Perforation	–	–	–	2 (4%)
Muscularis, degeneration	–	–	1 (2%)	–
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	–	–	3 (6%)
Ulcer	–	–	–	1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)
Parasite metazoan	–	1 (2%)	1 (2%)	–
Epithelium, necrosis	–	–	1 (2%)	1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)
Parasite metazoan	5 (10%)	1 (2%)	4 (8%)	–
Muscularis, hyperplasia	–	1 (2%)	–	–
Intestine small, duodenum	(50)	(47)	(49)	(48)
Epithelium, regeneration	–	–	1 (2%)	–
Mucosa, necrosis	–	1 (2%)	1 (2%)	10 (21%)
Intestine small, ileum	(50)	(48)	(49)	(45)
Mucosa, necrosis	–	1 (2%)	2 (4%)	6 (13%)
Intestine small, jejunum	(49)	(47)	(48)	(46)
Mucosa, necrosis	–	–	2 (4%)	9 (20%)
Muscularis, hyperplasia	–	–	1 (2%)	–
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)	1 (2%)	3 (6%)	–
Basophilic focus	20 (40%)	20 (40%)	33 (66%)	11 (22%)
Clear cell focus	28 (56%)	36 (72%)	34 (68%)	20 (40%)
Deformity	1 (2%)	–	–	–
Degeneration, cystic	–	–	–	1 (2%)
Eosinophilic focus	5 (10%)	5 (10%)	5 (10%)	–
Fatty change	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Hematopoietic cell proliferation	3 (6%)	–	1 (2%)	–
Hematopoietic cell proliferation, granulocytic	–	1 (2%)	–	–

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Hepatodiaphragmatic nodule	1 (2%)	–	1 (2%)	1 (2%)
Inflammation	3 (6%)	7 (14%)	5 (10%)	2 (4%)
Inflammation, chronic active	–	–	1 (2%)	–
Mixed cell focus	10 (20%)	9 (18%)	12 (24%)	7 (14%)
Necrosis	1 (2%)	2 (4%)	2 (4%)	13 (26%)
Pigmentation	–	2 (4%)	1 (2%)	1 (2%)
Vacuolization, cytoplasmic, focal	3 (6%)	15 (30%)	9 (18%)	1 (2%)
Bile duct, hyperplasia	11 (22%)	14 (28%)	6 (12%)	10 (20%)
Bile duct, hyperplasia, cystic	–	–	–	1 (2%)
Hepatocyte, atrophy	1 (2%)	–	–	–
Hepatocyte, regeneration	1 (2%)	–	–	–
Oval cell, hyperplasia	–	–	–	2 (4%)
Portal fibrosis	–	1 (2%)	–	–
Portal, infiltration cellular, mononuclear cell	–	–	2 (4%)	–
Mesentery	(3)	(2)	(1)	(1)
Inflammation	–	1 (50%)	–	–
Necrosis	2 (67%)	1 (50%)	–	1 (100%)
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus	1 (2%)	–	–	–
Lipomatosis	–	1 (2%)	–	–
Pigmentation, hemosiderin	–	–	1 (2%)	–
Acinus, atrophy	7 (14%)	6 (12%)	9 (18%)	5 (10%)
Acinus, hyperplasia	3 (6%)	–	–	–
Acinus, necrosis	–	–	–	1 (2%)
Salivary glands	(50)	(50)	(50)	(48)
Amyloid deposition	1 (2%)	–	–	–
Atrophy	1 (2%)	–	–	–
Necrosis	–	–	–	1 (2%)
Duct, cyst	–	–	1 (2%)	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion	2 (4%)	–	–	–
Inflammation	3 (6%)	2 (4%)	–	–
Ulcer	–	3 (6%)	–	2 (4%)
Epithelium, degeneration, hydropic	–	–	1 (2%)	–

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Epithelium, hyperkeratosis	1 (2%)	1 (2%)	–	–
Epithelium, hyperplasia	7 (14%)	6 (12%)	7 (14%)	8 (16%)
Stomach, glandular	(49)	(50)	(50)	(50)
Cyst, squamous	–	–	1 (2%)	–
Inflammation	2 (4%)	–	–	1 (2%)
Mineralization	1 (2%)	–	–	1 (2%)
Mucosa, hyalinization	–	–	1 (2%)	–
Mucosa, necrosis	–	3 (6%)	3 (6%)	21 (42%)
Mucosa, pigmentation	–	1 (2%)	–	–
Tooth	(1)	(1)	(0)	(0)
Inflammation	1 (100%)	–	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Adventitia, aorta, hemorrhage	–	–	1 (2%)	1 (2%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	45 (90%)	43 (86%)	40 (80%)	28 (56%)
Inflammation	–	–	1 (2%)	1 (2%)
Endocardium, hyperplasia	–	1 (2%)	–	–
Epicardium, inflammation	–	–	1 (2%)	5 (10%)
Myocardium, necrosis	–	–	–	3 (6%)
Pericardium, inflammation	–	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	–	1 (2%)	–	–
Degeneration, cystic	2 (4%)	1 (2%)	1 (2%)	–
Hematopoietic cell proliferation, granulocytic	1 (2%)	–	–	–
Hyperplasia	22 (44%)	22 (44%)	25 (50%)	8 (16%)
Hypertrophy	20 (40%)	24 (48%)	14 (28%)	6 (12%)
Infiltration cellular, lipocyte	–	1 (2%)	–	–
Necrosis	2 (4%)	–	–	5 (10%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	–	1 (2%)	2 (4%)	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(47)	(44)	(49)	(45)
Hyperplasia	2 (4%)	–	1 (2%)	1 (2%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Inflammation	–	–	–	1 (2%)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, atrophy	–	1 (2%)	–	–
Pars distalis, hyperplasia	12 (24%)	10 (20%)	13 (26%)	8 (16%)
Pars intermedia, hyperplasia	–	–	1 (2%)	–
Thyroid gland	(50)	(50)	(50)	(49)
Amyloid deposition	–	–	1 (2%)	–
Hemorrhage	–	–	–	1 (2%)
C-cell, hyperplasia	37 (74%)	30 (60%)	30 (60%)	15 (31%)
Follicle, cyst	1 (2%)	–	–	–
Follicular cell, hyperplasia	–	1 (2%)	3 (6%)	–
General Body System				
None	–	–	–	–
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)	–	1 (2%)	–
Spermatocele	1 (2%)	–	–	–
Preputial gland	(50)	(50)	(50)	(50)
Inflammation	–	1 (2%)	–	–
Prostate	(50)	(50)	(50)	(50)
Inflammation	3 (6%)	4 (8%)	–	2 (4%)
Epithelium, hyperplasia	6 (12%)	13 (26%)	6 (12%)	8 (16%)
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	5 (10%)	–	2 (4%)
Epithelium, hyperplasia	1 (2%)	2 (4%)	–	–
Testes	(50)	(50)	(50)	(50)
Atrophy	9 (18%)	13 (26%)	13 (26%)	5 (10%)
Degeneration, cystic	3 (6%)	7 (14%)	7 (14%)	–
Mineralization	–	–	–	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	10 (20%)	9 (18%)	11 (22%)	13 (26%)
Hyperplasia, histiocyte	–	1 (2%)	–	–
Lymph node	(4)	(0)	(1)	(1)
Mediastinal, degeneration, cystic	1 (25%)	–	–	–
Mediastinal, hemorrhage	1 (25%)	–	–	–

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Mediastinal, hyperplasia, lymphoid	2 (50%)	–	–	1 (100%)
Lymph node, mandibular	(49)	(50)	(50)	(48)
Degeneration, cystic	5 (10%)	8 (16%)	4 (8%)	3 (6%)
Hemorrhage	–	–	1 (2%)	–
Hyperplasia, lymphoid	–	–	–	1 (2%)
Hyperplasia, plasma cell	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy	–	–	–	1 (2%)
Degeneration, cystic	2 (4%)	–	1 (2%)	1 (2%)
Hemorrhage	–	–	1 (2%)	–
Spleen	(50)	(50)	(50)	(50)
Angiectasis	–	1 (2%)	–	–
Depletion lymphoid	1 (2%)	2 (4%)	1 (2%)	13 (26%)
Hematopoietic cell proliferation	7 (14%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)	–	1 (2%)	–
Thymus	(49)	(49)	(50)	(50)
Atrophy	–	–	–	1 (2%)
Hemorrhage	1 (2%)	–	–	–
Hyperplasia	–	–	–	1 (2%)
Inflammation	–	–	–	1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Duct, dilatation	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	3 (6%)	3 (6%)	1 (2%)	–
Hyperplasia	2 (4%)	1 (2%)	–	–
Inflammation	2 (4%)	–	–	–
Ulcer	8 (16%)	4 (8%)	2 (4%)	–
Hair follicle, cyst epithelial inclusion, multiple	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(0)	(0)	(1)
Cyst	–	–	–	1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Ventricle, developmental malformation	–	1 (2%)	–	–
Peripheral nerve	(0)	(1)	(0)	(1)
Spinal cord	(0)	(1)	(0)	(1)
Degeneration	–	1 (100%)	–	–
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	–	–	–	2 (4%)
Inflammation, suppurative	–	1 (2%)	3 (6%)	10 (20%)
Inflammation, chronic active	10 (20%)	6 (12%)	7 (14%)	11 (22%)
Mineralization	1 (2%)	–	–	–
Alveolar epithelium, hyperplasia	2 (4%)	4 (8%)	5 (10%)	5 (10%)
Alveolar epithelium, metaplasia	–	–	–	1 (2%)
Alveolus, infiltration cellular, histiocyte	23 (46%)	21 (42%)	28 (56%)	25 (50%)
Bronchiole, hyperplasia	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Foreign body	8 (16%)	5 (10%)	4 (8%)	5 (10%)
Inflammation, suppurative	11 (22%)	12 (24%)	20 (40%)	42 (84%)
Epithelium, goblet cell, nasopharyngeal duct, hyperplasia	1 (2%)	1 (2%)	1 (2%)	–
Epithelium, nasopharyngeal duct, degeneration	–	1 (2%)	–	3 (6%)
Epithelium, nasopharyngeal duct, hyperplasia	–	–	2 (4%)	4 (8%)
Epithelium, nasopharyngeal duct, metaplasia, squamous	–	1 (2%)	–	1 (2%)
Epithelium, nasopharyngeal duct, necrosis	–	–	–	2 (4%)
Epithelium, nasopharyngeal duct, pigmentation	–	–	–	1 (2%)
Epithelium, nasopharyngeal duct, regeneration	–	–	2 (4%)	2 (4%)
Goblet cell, nasopharyngeal duct, hyperplasia	–	1 (2%)	–	–
Lamina propria, mineralization	–	33 (66%)	34 (68%)	44 (88%)
Lamina propria, pigmentation	–	4 (8%)	11 (22%)	25 (50%)
Lumen, pigmentation, histiocyte	–	1 (2%)	–	–
Nasopharyngeal duct, foreign body	–	2 (4%)	1 (2%)	2 (4%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Nasopharyngeal duct, inflammation, suppurative	–	6 (12%)	8 (16%)	20 (40%)
Nasopharyngeal duct, mineralization	–	–	–	1 (2%)
Nerve, atrophy	–	33 (66%)	44 (88%)	44 (88%)
Olfactory epithelium, accumulation, hyaline droplet	31 (62%)	30 (60%)	22 (44%)	4 (8%)
Olfactory epithelium, atrophy	1 (2%)	38 (76%)	41 (82%)	41 (82%)
Olfactory epithelium, hyperplasia, basal cell	–	1 (2%)	9 (18%)	28 (56%)
Olfactory epithelium, metaplasia, respiratory	4 (8%)	40 (80%)	43 (86%)	47 (94%)
Olfactory epithelium, necrosis	1 (2%)	3 (6%)	–	12 (24%)
Olfactory epithelium, pigmentation	6 (12%)	18 (36%)	12 (24%)	21 (42%)
Olfactory epithelium, squamous metaplasia	–	–	1 (2%)	4 (8%)
Olfactory epithelium, ulcer	–	–	–	1 (2%)
Respiratory epithelium, accumulation, hyaline droplet	23 (46%)	30 (60%)	29 (58%)	10 (20%)
Respiratory epithelium, atrophy	–	2 (4%)	5 (10%)	6 (12%)
Respiratory epithelium, degeneration	–	–	–	3 (6%)
Respiratory epithelium, hyperplasia	1 (2%)	1 (2%)	4 (8%)	5 (10%)
Respiratory epithelium, metaplasia, squamous	–	1 (2%)	3 (6%)	7 (14%)
Respiratory epithelium, necrosis	–	–	–	4 (8%)
Respiratory epithelium, pigmentation	2 (4%)	6 (12%)	7 (14%)	7 (14%)
Turbinate, deformity	–	16 (32%)	22 (44%)	35 (70%)
Turbinate, hyperostosis	–	18 (36%)	27 (54%)	40 (80%)
Trachea	(50)	(50)	(50)	(50)
Inflammation	–	–	–	3 (6%)
Epithelium, hyperplasia	–	–	1 (2%)	–
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	–	–	1 (2%)	1 (2%)
Synechia	–	1 (2%)	1 (2%)	–
Ciliary body, inflammation	–	1 (2%)	–	–

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Cornea, inflammation	1 (2%)	–	–	–
Retina, atrophy	1 (2%)	–	2 (4%)	1 (2%)
Retina, dysplasia	–	1 (2%)	–	–
Retina, hyperplasia, reticulum cell	1 (2%)	–	–	–
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	–	–	1 (2%)	–
Inflammation	1 (2%)	1 (2%)	–	1 (2%)
Zymbal's gland	(1)	(0)	(0)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	–	–	–	1 (2%)
Casts granular	–	–	–	1 (2%)
Cyst	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Hydronephrosis	2 (4%)	2 (4%)	2 (4%)	4 (8%)
Infarct	–	–	–	2 (4%)
Infiltration cellular, lipocyte	–	–	–	1 (2%)
Inflammation	14 (28%)	10 (20%)	13 (26%)	10 (20%)
Nephropathy	47 (94%)	40 (80%)	44 (88%)	28 (56%)
Papilla, necrosis	2 (4%)	–	–	1 (2%)
Renal tubule, hyperplasia	–	–	2 (4%)	–
Transitional epithelium, hyperplasia	1 (2%)	4 (8%)	1 (2%)	2 (4%)
Ureter	(1)	(0)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, hyperplasia	–	–	–	1 (2%)

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

Appendix B. Summary of Lesions in Female Wistar Han Rats in the Two-year Gavage Study of Green Tea Extract

Tables

Table B-1. Summary of the Incidence of Neoplasms in Female Rats in the Two-year Gavage Study of Green Tea Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
Three-month interim evaluation	10	–	–	10
Early deaths				
Accidental deaths	–	–	1	3
Moribund	14	12	17	8
Natural deaths	10	10	9	35
Survivors				
Terminal kill	26	28	23	4
Animals examined microscopically	60	50	50	60
Systems Examined at Three Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
Two-year Study				
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Fibrosarcoma	–	1 (2%)	–	–
Intestine large, cecum	(49)	(49)	(48)	(44)
Sarcoma, metastatic, uterus	–	–	1 (2%)	–
Intestine large, colon	(49)	(49)	(48)	(44)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–
Sarcoma, metastatic, uterus	–	–	1 (2%)	–
Intestine large, rectum	(49)	(49)	(50)	(45)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Intestine small, duodenum	(47)	(48)	(48)	(39)
Sarcoma, metastatic, uterus	–	–	1 (2%)	–
Serosa, adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–
Intestine small, ileum	(45)	(46)	(47)	(36)
Serosa, adenocarcinoma, metastatic, uterus	–	–	1 (2%)	–
Intestine small, jejunum	(45)	(43)	(45)	(40)
Leiomyosarcoma	1 (2%)	1 (2%)	–	–
Sarcoma, metastatic, uterus	–	–	1 (2%)	–
Liver	(50)	(48)	(49)	(46)
Adenocarcinoma, metastatic, uterus	–	2 (4%)	1 (2%)	–
Carcinoma, metastatic, pancreas	–	–	–	2 (4%)
Cholangioma	–	1 (2%)	–	–
Hepatocellular adenoma	1 (2%)	2 (4%)	–	2 (4%)
Sarcoma, metastatic, uterus	–	–	1 (2%)	–
Capsule, adenocarcinoma, metastatic, uterus	–	–	1 (2%)	–
Mesentery	(6)	(3)	(3)	(2)
Adenocarcinoma, metastatic, uterus	1 (17%)	3 (100%)	2 (67%)	–
Carcinoma, metastatic, pancreas	–	–	–	1 (50%)
Hemangiosarcoma	1 (17%)	–	–	–
Oral mucosa	(1)	(0)	(0)	(0)
Squamous cell carcinoma	1 (100%)	–	–	–
Pancreas	(50)	(49)	(48)	(44)
Adenocarcinoma, metastatic, uterus	1 (2%)	1 (2%)	1 (2%)	–
Carcinoma	–	–	–	2 (5%)
Carcinoma, metastatic, uterus	–	1 (2%)	–	–
Salivary glands	(49)	(48)	(50)	(46)
Stomach, forestomach	(50)	(50)	(49)	(44)
Adenocarcinoma, metastatic, uterus	–	2 (4%)	1 (2%)	–
Leiomyosarcoma	–	1 (2%)	–	–
Squamous cell carcinoma	1 (2%)	–	–	–
Stomach, glandular	(50)	(49)	(49)	(44)
Adenocarcinoma, metastatic, uterus	–	2 (4%)	–	–
Carcinoma, metastatic, uterus	1 (2%)	–	–	–
Fibrosarcoma	–	–	–	1 (2%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Cardiovascular System				
Blood vessel	(50)	(48)	(50)	(46)
Aorta, sarcoma, metastatic, uterus	–	–	1 (2%)	–
Heart	(50)	(48)	(50)	(48)
Sarcoma, metastatic, uterus	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(49)	(49)	(47)
Adenocarcinoma, metastatic, uterus	–	–	1 (2%)	–
Adenoma	–	2 (4%)	1 (2%)	–
Capsule, adenocarcinoma, metastatic, uterus	–	–	1 (2%)	–
Adrenal medulla	(50)	(49)	(49)	(47)
Pheochromocytoma benign	1 (2%)	–	2 (4%)	–
Islets, pancreatic	(50)	(49)	(48)	(44)
Parathyroid gland	(47)	(46)	(48)	(44)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	29 (58%)	17 (34%)	12 (24%)	6 (12%)
Pars distalis, adenoma, multiple	3 (6%)	4 (8%)	1 (2%)	2 (4%)
Pars distalis, carcinoma	–	–	1 (2%)	–
Pars intermedia, adenoma	1 (2%)	–	2 (4%)	–
Pars intermedia, adenoma, multiple	1 (2%)	–	–	–
Pars nervosa, schwannoma malignant	–	–	–	1 (2%)
Thyroid gland	(50)	(49)	(50)	(47)
Sarcoma, metastatic, uterus	–	–	1 (2%)	–
Bilateral, C-cell, adenoma	–	1 (2%)	–	–
C-cell, adenoma	3 (6%)	3 (6%)	4 (8%)	1 (2%)
C-cell, carcinoma	1 (2%)	–	–	–
Follicular cell, adenoma	1 (2%)	–	4 (8%)	1 (2%)
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(49)	(49)	(50)	(46)
Adenoma	–	–	1 (2%)	–
Carcinoma	–	–	1 (2%)	–
Sarcoma	–	–	1 (2%)	–
Ovary	(50)	(49)	(49)	(44)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Adenocarcinoma, metastatic, uterus	–	1 (2%)	2 (4%)	–
Carcinoma, metastatic, pancreas	–	–	–	1 (2%)
Sarcoma stromal, metastatic, uterus	–	–	1 (2%)	–
Oviduct	(1)	(0)	(0)	(0)
Uterus	(50)	(49)	(49)	(44)
Adenocarcinoma	3 (6%)	9 (18%)	5 (10%)	3 (7%)
Adenocarcinoma, squamous differentiation	–	–	–	1 (2%)
Adenoma	–	–	–	1 (2%)
Polyp stromal	2 (4%)	1 (2%)	1 (2%)	3 (7%)
Sarcoma stromal	–	1 (2%)	2 (4%)	–
Schwannoma malignant	1 (2%)	–	2 (4%)	1 (2%)
Cervix, polyp stromal	1 (2%)	–	–	–
Cervix, sarcoma stromal	1 (2%)	–	–	–
Cervix, schwannoma malignant	–	–	–	1 (2%)
Vagina	(1)	(2)	(0)	(0)
Adenocarcinoma, metastatic, uterus	–	1 (50%)	–	–
Polyp	1 (100%)	–	–	–
Sarcoma stromal	–	1 (50%)	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(3)	(1)	(4)	(6)
Iliac, adenocarcinoma, metastatic, uterus	1 (33%)	–	–	–
Renal, carcinoma, metastatic, uncertain primary site	–	–	–	1 (17%)
Lymph node, mandibular	(49)	(48)	(50)	(46)
Carcinoma, metastatic, Zymbal's gland	–	–	1 (2%)	–
Lymph node, mesenteric	(50)	(49)	(48)	(45)
Adenocarcinoma, metastatic, uterus	1 (2%)	1 (2%)	1 (2%)	–
Hemangiosarcoma	1 (2%)	–	–	–
Spleen	(50)	(49)	(48)	(43)
Adenocarcinoma, metastatic, uterus	–	2 (4%)	2 (4%)	–
Carcinoma, metastatic, pancreas	–	–	–	1 (2%)
Thymus	(49)	(47)	(50)	(47)
Sarcoma, metastatic, uterus	–	–	1 (2%)	–
Thymoma benign	3 (6%)	3 (6%)	5 (10%)	–

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Integumentary System				
Mammary gland	(50)	(49)	(49)	(47)
Adenoma	2 (4%)	–	1 (2%)	–
Carcinoma	5 (10%)	–	4 (8%)	–
Carcinoma, multiple	1 (2%)	3 (6%)	1 (2%)	–
Fibroadenoma	10 (20%)	7 (14%)	5 (10%)	1 (2%)
Fibroadenoma, multiple	1 (2%)	1 (2%)	–	–
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	–	1 (2%)	–	–
Trichoepithelioma	–	1 (2%)	–	–
Subcutaneous tissue, lipoma	–	1 (2%)	1 (2%)	–
Subcutaneous tissue, schwannoma malignant	2 (4%)	–	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(49)
Skeletal muscle	(0)	(0)	(3)	(2)
Adenocarcinoma, metastatic, uterus	–	–	2 (67%)	–
Carcinoma, metastatic, pancreas	–	–	–	2 (100%)
Carcinoma, metastatic, Zymbal's gland	–	–	1 (33%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma malignant	–	1 (2%)	–	–
Granular cell tumor benign	–	–	–	1 (2%)
Oligodendroglioma malignant	–	1 (2%)	–	–
Meninges, schwannoma malignant, metastatic, pituitary gland	–	–	–	1 (2%)
Peripheral nerve	(1)	(2)	(0)	(0)
Spinal cord	(1)	(2)	(0)	(0)
Respiratory System				
Lung	(50)	(49)	(50)	(48)
Adenocarcinoma, metastatic, uterus	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Carcinoma, metastatic, mammary gland	–	–	1 (2%)	–
Carcinoma, metastatic, pancreas	–	–	–	1 (2%)
Carcinoma, metastatic, uterus	1 (2%)	–	–	–
Sarcoma stromal, metastatic, uterus	–	–	2 (4%)	–

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Nose	(49)	(49)	(50)	(49)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(48)	(50)	(50)	(49)
Sarcoma, stromal, metastatic, uterus	–	–	1 (2%)	–
Harderian gland	(48)	(50)	(50)	(49)
Zymbal's gland	(0)	(0)	(1)	(0)
Carcinoma	–	–	1 (100%)	–
Urinary System				
Kidney	(50)	(49)	(49)	(45)
Adenocarcinoma, metastatic, uterus	–	–	1 (2%)	–
Lipoma	1 (2%)	–	–	–
Capsule, adenocarcinoma, metastatic, uterus	–	–	1 (2%)	–
Urinary bladder	(49)	(49)	(49)	(44)
Adenocarcinoma, metastatic, uterus	–	–	1 (2%)	–
Carcinoma, metastatic, pancreas	–	–	–	1 (2%)
Carcinoma, metastatic, uterus	–	1 (2%)	–	–
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	–	–	1 (2%)
Lymphoma malignant	1 (2%)	–	–	–
Neoplasm Summary				
Total animals with primary neoplasms[c]				
Two-year study	43	37	31	18
Total primary neoplasms				
Two-year study	81	64	58	29
Total animals with benign neoplasms				
Two-year study	38	31	25	15
Total benign neoplasms				
Two-year study	61	44	40	18
Total animals with malignant neoplasms				
Two-year study	16	19	17	8
Total malignant neoplasms				
Two-year study	20	20	18	11

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Total animals with metastatic neoplasms				
Two-year study	2	3	6	5
Total metastatic neoplasms				
Two-year study	7	22	37	12
Total animals with malignant neoplasms of uncertain primary site				
Two-year study	–	–	–	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 B-2. Statistical Analysis of Primary Neoplasms in Female Rats in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Mammary Gland: Fibroadenoma				
Overall rate[a]	11/50 (22%)	8/50 (16%)	5/50 (10%)	1/50 (2%)
Adjusted rate[b]	26.2%	19.8%	14.6%	6.0%
Terminal rate[c]	7/26 (27%)	5/28 (18%)	1/23 (4%)	0/4 (0%)
First incidence (days)	561	628	506	533
Poly-3 test[d]	P = 0.070N	P = 0.336N	P = 0.171N	P = 0.123N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	11/50 (22%)	8/50 (16%)	6/50 (12%)	1/50 (2%)
Adjusted rate	26.2%	19.8%	17.5%	6.0%
Terminal rate	7/26 (27%)	5/28 (18%)	1/23 (4%)	0/4 (0%)
First incidence (days)	561	628	506	533
Poly-3 test	P = 0.092N	P = 0.336N	P = 0.265N	P = 0.123N
Mammary Gland: Carcinoma				
Overall rate	6/50 (12%)	3/50 (6%)	5/50 (10%)	0/50 (0%)
Adjusted rate	14.5%	7.5%	14.3%	0.0%
Terminal rate	4/26 (15%)	2/28 (7%)	1/23 (4%)	0/4 (0%)
First incidence (days)	667	694	275	–[e]
Poly-3 test	P = 0.236N	P = 0.257N	P = 0.615N	P = 0.173N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	3/50 (6%)	5/50 (10%)	0/50 (0%)
Adjusted rate	19.4%	7.5%	14.3%	0.0%
Terminal rate	6/26 (23%)	2/28 (7%)	1/23 (4%)	0/4 (0%)
First incidence (days)	667	694	275	–
Poly-3 test	P = 0.127N	P = 0.106N	P = 0.388N	P = 0.108N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	15/50 (30%)	11/50 (22%)	9/50 (18%)	1/50 (2%)
Adjusted rate	35.5%	27.1%	24.9%	6.0%
Terminal rate	10/26 (39%)	7/28 (25%)	2/23 (9%)	0/4 (0%)
First incidence (days)	561	628	275	533
Poly-3 test	P = 0.040N	P = 0.279N	P = 0.220N	P = 0.045N
Pancreas: Carcinoma				
Overall rate	0/50 (0%)	0/49 (0%)	0/48 (0%)	2/44 (5%)
Adjusted rate	0.0%	0.0%	0.0%	12.0%
Terminal rate	0/26 (0%)	0/28 (0%)	0/23 (0%)	0/4 (0%)
First incidence (days)	–	–	–	540
Poly-3 test	P = 0.017	–[f]	–	P = 0.102
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	32/50 (64%)	21/50 (42%)	13/50 (26%)	8/50 (16%)
Adjusted rate	68.9%	50.4%	38.1%	40.5%

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Terminal rate	16/26 (62%)	14/28 (50%)	8/23 (35%)	0/4 (0%)
First incidence (days)	495	506	530	513
Poly-3 test	P = 0.018N	P = 0.054N	P = 0.004N	P = 0.042N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	32/50 (64%)	21/50 (42%)	14/50 (28%)	8/50 (16%)
Adjusted rate	68.9%	50.4%	41.0%	40.5%
Terminal rate	16/26 (62%)	14/28 (50%)	9/23 (39%)	0/4 (0%)
First incidence (days)	495	506	530	513
Poly-3 test	P = 0.022N	P = 0.054N	P = 0.009N	P = 0.042N
Thymus: Benign Thymoma				
Overall rate	3/49 (6%)	3/47 (6%)	5/50 (10%)	0/47 (0%)
Adjusted rate	7.4%	7.9%	15.0%	0.0%
Terminal rate	3/26 (12%)	3/28 (11%)	4/23 (17%)	0/4 (0%)
First incidence (days)	729 (T)	729 (T)	530	–
Poly-3 test	P = 0.493N	P = 0.630	P = 0.254	P = 0.357N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	3/50 (6%)	4/49 (8%)	4/50 (8%)	1/47 (2%)
Adjusted rate	7.3%	10.2%	12.0%	6.5%
Terminal rate	3/26 (12%)	3/28 (11%)	3/23 (13%)	0/4 (0%)
First incidence (days)	729 (T)	719	584	714
Poly-3 test	P = 0.531	P = 0.478	P = 0.388	P = 0.649N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	4/50 (8%)	4/49 (8%)	4/50 (8%)	1/47 (2%)
Adjusted rate	9.8%	10.2%	12.0%	6.5%
Terminal rate	4/26 (15%)	3/28 (11%)	3/23 (13%)	0/4 (0%)
First incidence (days)	729 (T)	719	584	714
Poly-3 test	P = 0.508N	P = 0.623	P = 0.526	P = 0.543N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	1/50 (2%)	0/49 (0%)	4/50 (8%)	1/47 (2%)
Adjusted rate	2.4%	0.0%	12.1%	6.2%
Terminal rate	1/26 (4%)	0/28 (0%)	3/23 (13%)	0/4 (0%)
First incidence (days)	729 (T)	–	675	513
Poly-3 test	P = 0.146	P = 0.508N	P = 0.120	P = 0.534
Uterus: Stromal Polyp				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	7.2%	2.5%	3.1%	17.3%
Terminal rate	1/26 (4%)	1/28 (4%)	1/23 (4%)	1/4 (25%)
First incidence (days)	561	729 (T)	729 (T)	509
Poly-3 test	P = 0.201	P = 0.321N	P = 0.394N	P = 0.278
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)	3/50 (6%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Adjusted rate	9.5%	5.0%	9.0%	17.3%
Terminal rate	1/26 (4%)	2/28 (7%)	2/23 (9%)	1/4 (25%)
First incidence (days)	561	729 (T)	584	509
Poly-3 test	P = 0.253	P = 0.364N	P = 0.626N	P = 0.366
Uterus: Adenocarcinoma				
Overall rate	3/50 (6%)	9/50 (18%)	5/50 (10%)	4/50 (8%)
Adjusted rate	7.3%	21.9%	14.9%	23.0%
Terminal rate	1/26 (4%)	4/28 (14%)	2/23 (9%)	1/4 (25%)
First incidence (days)	688	484	610	533
Poly-3 test	P = 0.202	P = 0.056	P = 0.248	P = 0.141
All Organs: Benign Neoplasms				
Overall rate	38/50 (76%)	31/50 (62%)	25/50 (50%)	15/50 (30%)
Adjusted rate	81.6%	73.2%	69.1%	67.5%
Terminal rate	21/26 (81%)	20/28 (71%)	14/23 (61%)	2/4 (50%)
First incidence (days)	495	506	506	509
Poly-3 test	P = 0.139N	P = 0.232N	P = 0.135N	P = 0.163N
All Organs: Malignant Neoplasms				
Overall rate	16/50 (32%)	19/50 (38%)	17/50 (34%)	9/50 (18%)
Adjusted rate	36.3%	44.0%	44.3%	44.2%
Terminal rate	6/26 (23%)	9/28 (32%)	6/23 (26%)	1/4 (25%)
First incidence (days)	506	484	275	434
Poly-3 test	P = 0.347	P = 0.301	P = 0.302	P = 0.385
All Organs: Benign or Malignant Neoplasms				
Overall rate	43/50 (86%)	37/50 (74%)	31/50 (62%)	19/50 (38%)
Adjusted rate	89.2%	83.0%	78.8%	79.6%
Terminal rate	21/26 (81%)	22/28 (79%)	16/23 (70%)	3/4 (75%)
First incidence (days)	495	484	275	434
Poly-3 test	P = 0.188N	P = 0.282N	P = 0.145N	P = 0.227N

T = terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for pancreas, pituitary gland, thymus, 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 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 B-3. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Two-year Gavage Study of Green Tea Extract[a]

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	60	50	50	60
Three-month interim evaluation	10	–	–	10
Early deaths				
Accidental deaths	–	–	1	3
Moribund	14	12	17	8
Natural deaths	10	10	9	35
Survivors				
Terminal kill	26	28	23	4
Animals examined microscopically	60	50	50	60
Three-month interim evaluation				
Alimentary System				
Liver	(10)	(0)	(0)	(10)
Basophilic focus	–	–	–	1 (10%)
Inflammation	–	–	–	3 (30%)
Necrosis	–	–	–	1 (10%)
Bile duct, hyperplasia	–	–	–	1 (10%)
Hepatocyte, hypertrophy	–	–	–	1 (10%)
Oval cell, hyperplasia	–	–	–	1 (10%)
Salivary glands	(10)	(0)	(0)	(10)
Inflammation	1 (10%)	–	–	–
Cardiovascular System				
Heart	(10)	(0)	(0)	(10)
Cardiomyopathy	–	–	–	1 (10%)
Genital System				
Clitoral gland	(10)	(0)	(0)	(10)
Inflammation	2 (20%)	–	–	2 (20%)
Uterus	(10)	(0)	(0)	(10)
Endometrium, hyperplasia, cystic	1 (10%)	–	–	1 (10%)
Hematopoietic System				
Lymph node, mandibular	(10)	(0)	(0)	(10)
Atrophy	–	–	–	1 (10%)
Hyperplasia, plasma cell	6 (60%)	–	–	5 (50%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Respiratory System				
Lung	(10)	(0)	(0)	(10)
Inflammation, chronic active	2 (20%)	–	–	2 (20%)
Nose	(10)	(0)	(0)	(10)
Inflammation, suppurative	–	–	–	1 (10%)
Inflammation, acute	–	–	–	1 (10%)
Epithelium, nasopharyngeal duct, degeneration	–	–	–	1 (10%)
Epithelium, nasopharyngeal duct, regeneration	–	–	–	1 (10%)
Lamina propria, pigmentation	–	–	–	1 (10%)
Nasopharyngeal duct, inflammation, acute	–	–	–	1 (10%)
Nerve, atrophy	–	–	–	1 (10%)
Olfactory epithelium, accumulation, hyaline droplet	–	–	–	1 (10%)
Olfactory epithelium, atrophy	–	–	–	2 (20%)
Olfactory epithelium, metaplasia, respiratory	1 (10%)	–	–	4 (40%)
Olfactory epithelium, pigmentation	–	–	–	4 (40%)
Respiratory epithelium, accumulation, hyaline droplet	–	–	–	1 (10%)
Respiratory epithelium, degeneration	1 (10%)	–	–	–
Respiratory epithelium, hyperplasia	–	–	–	2 (20%)
Turbinate, malformation	–	–	–	1 (10%)
Trachea	(10)	(0)	(0)	(10)
Glands, cyst	1 (10%)	–	–	–
Urinary System				
Kidney	(10)	(0)	(0)	(10)
Cyst	1 (10%)	–	–	–
Pelvis, dilatation	1 (10%)	–	–	–
Systems examined at 3 months with no lesions observed				
Endocrine System				
General Body System				
Integumentary System				

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Musculoskeletal System				
Nervous System				
Special Senses System				
Two-year Study				
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	–	1 (2%)	–	–
Perforation	–	–	–	1 (2%)
Muscularis, degeneration	1 (2%)	–	–	–
Intestine large, cecum	(49)	(49)	(48)	(44)
Inflammation	1 (2%)	–	1 (2%)	1 (2%)
Lymphoid tissue, hyperplasia	1 (2%)	1 (2%)	–	–
Intestine large, colon	(49)	(49)	(48)	(44)
Hyperplasia, lymphoid	–	–	1 (2%)	–
Inflammation	–	–	–	1 (2%)
Parasite, metazoan	1 (2%)	–	1 (2%)	–
Intestine large, rectum	(49)	(49)	(50)	(45)
Inflammation	–	–	1 (2%)	–
Parasite metazoan	1 (2%)	–	–	1 (2%)
Thrombosis	–	–	–	1 (2%)
Intestine small, duodenum	(47)	(48)	(48)	(39)
Mucosa, necrosis	–	–	1 (2%)	5 (13%)
Intestine small, ileum	(45)	(46)	(47)	(36)
Mucosa, necrosis	–	–	–	5 (14%)
Serosa, hemorrhage	–	–	1 (2%)	–
Serosa, inflammation	–	–	1 (2%)	–
Intestine small, jejunum	(45)	(43)	(45)	(40)
Necrosis, hemorrhagic	–	1 (2%)	–	–
Mucosa, necrosis	–	–	1 (2%)	6 (15%)
Liver	(50)	(48)	(49)	(46)
Angiectasis	1 (2%)	4 (8%)	–	–
Basophilic focus	38 (76%)	38 (79%)	35 (71%)	15 (33%)
Clear cell focus	14 (28%)	8 (17%)	6 (12%)	5 (11%)
Degeneration, cystic	1 (2%)	–	–	–
Eosinophilic focus	3 (6%)	4 (8%)	1 (2%)	4 (9%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Fatty change	7 (14%)	1 (2%)	–	–
Hematopoietic cell proliferation	–	4 (8%)	5 (10%)	–
Hematopoietic cell proliferation, granulocytic	–	3 (6%)	2 (4%)	1 (2%)
Hepatodiaphragmatic nodule	–	–	1 (2%)	–
Inflammation	5 (10%)	3 (6%)	4 (8%)	4 (9%)
Mixed cell focus	7 (14%)	4 (8%)	1 (2%)	2 (4%)
Necrosis	3 (6%)	2 (4%)	5 (10%)	24 (52%)
Pigmentation	1 (2%)	1 (2%)	2 (4%)	5 (11%)
Vacuolization, cytoplasmic, focal	6 (12%)	2 (4%)	4 (8%)	1 (2%)
Bile duct, concretion	–	–	1 (2%)	–
Bile duct, cyst	–	2 (4%)	2 (4%)	–
Bile duct, dilatation	–	–	1 (2%)	–
Bile duct, hyperplasia	21 (42%)	11 (23%)	13 (27%)	17 (37%)
Bile duct, hyperplasia, cystic	1 (2%)	–	–	–
Hepatocyte, hyperplasia, regenerative	–	–	–	2 (4%)
Hepatocyte, regeneration	1 (2%)	–	–	–
Oval cell, hyperplasia	1 (2%)	2 (4%)	3 (6%)	16 (35%)
Portal infiltration cellular, mononuclear cell	2 (4%)	1 (2%)	–	–
Serosa, fibrosis	–	–	1 (2%)	–
Serosa, inflammation	–	–	1 (2%)	–
Mesentery	(6)	(3)	(3)	(2)
Degeneration, cystic	–	–	1 (33%)	–
Necrosis	4 (67%)	–	–	–
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(50)	(49)	(48)	(44)
Inflammation	1 (2%)	1 (2%)	2 (4%)	–
Lipomatosis	–	–	1 (2%)	–
Acinus, atrophy	3 (6%)	4 (8%)	2 (4%)	2 (5%)
Acinus, cytoplasmic alteration	–	1 (2%)	–	–
Duct, cyst	–	1 (2%)	–	–
Salivary glands	(49)	(48)	(50)	(46)
Duct, cyst	–	1 (2%)	–	–
Stomach, forestomach	(50)	(50)	(49)	(44)
Erosion	2 (4%)	2 (4%)	–	1 (2%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Inflammation	5 (10%)	1 (2%)	–	2 (5%)
Mineralization	–	1 (2%)	1 (2%)	2 (5%)
Ulcer	2 (4%)	1 (2%)	2 (4%)	2 (5%)
Epithelium, hyperkeratosis	1 (2%)	–	–	–
Epithelium, hyperplasia	3 (6%)	2 (4%)	1 (2%)	2 (5%)
Stomach, glandular	(50)	(49)	(49)	(44)
Inflammation	4 (8%)	1 (2%)	1 (2%)	2 (5%)
Mineralization	1 (2%)	2 (4%)	2 (4%)	2 (5%)
Necrosis	–	1 (2%)	–	–
Epithelium, degeneration	1 (2%)	–	–	–
Epithelium, hyperplasia	1 (2%)	–	2 (4%)	–
Glands, hyperplasia	–	–	–	1 (2%)
Mucosa, necrosis	–	1 (2%)	7 (14%)	20 (45%)
Cardiovascular System				
Blood vessel	(50)	(48)	(50)	(46)
Adventitia, aorta, hemorrhage	–	–	2 (4%)	1 (2%)
Heart	(50)	(48)	(50)	(48)
Cardiomyopathy	20 (40%)	24 (50%)	18 (36%)	11 (23%)
Inflammation	1 (2%)	–	1 (2%)	2 (4%)
Mineralization	–	–	–	1 (2%)
Endocardium, fibrosis	–	–	–	1 (2%)
Endocardium, hyperplasia	–	–	–	1 (2%)
Epicardium, inflammation	–	2 (4%)	2 (4%)	4 (8%)
Endocrine System				
Adrenal cortex	(50)	(49)	(49)	(47)
Angiectasis	–	–	2 (4%)	–
Atrophy	–	–	1 (2%)	–
Degeneration, cystic	1 (2%)	–	–	1 (2%)
Hemorrhage	–	–	1 (2%)	1 (2%)
Hyperplasia	21 (42%)	25 (51%)	27 (55%)	7 (15%)
Hypertrophy	13 (26%)	15 (31%)	10 (20%)	4 (9%)
Mineralization	–	–	1 (2%)	–
Necrosis	3 (6%)	–	–	3 (6%)
Adrenal medulla	(50)	(49)	(49)	(47)
Hyperplasia	2 (4%)	–	–	–
Vacuolization, cytoplasmic	–	2 (4%)	–	–

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Islets, pancreatic	(50)	(49)	(48)	(44)
Parathyroid gland	(47)	(46)	(48)	(44)
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	–	1 (2%)	–	–
Pars distalis, angiectasis	–	–	–	1 (2%)
Pars distalis, cyst	1 (2%)	–	–	
Pars distalis, hyperplasia	12 (24%)	13 (26%)	17 (34%)	13 (26%)
Pars intermedia, angiectasis	–	1 (2%)	–	1 (2%)
Pars intermedia, hyperplasia	1 (2%)	–	–	–
Thyroid gland	(50)	(49)	(50)	(47)
C-cell, hyperplasia	29 (58%)	14 (29%)	6 (12%)	4 (9%)
Follicle cell, hyperplasia	2 (4%)	1 (2%)	–	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(49)	(49)	(50)	(46)
Inflammation	2 (4%)	2 (4%)	–	–
Duct, cyst	–	–	1 (2%)	–
Ovary	(50)	(49)	(49)	(44)
Cyst	8 (16%)	12 (24%)	10 (20%)	6 (14%)
Inflammation	1 (2%)	5 (10%)	3 (6%)	6 (14%)
Oviduct	(1)	(0)	(0)	(0)
Uterus	(50)	(49)	(49)	(44)
Congestion	–	–	–	1 (2%)
Decidual reaction	–	1 (2%)	–	–
Hemorrhage	1 (2%)	–	–	–
Infiltration cellular, plasma cell	–	–	–	1 (2%)
Inflammation	3 (6%)	4 (8%)	2 (4%)	5 (11%)
Cervix, fibrosis	1 (2%)	–	–	–
Endometrium, hyperplasia, cystic	9 (18%)	14 (29%)	15 (31%)	9 (20%)
Vagina	(1)	(2)	(0)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	6 (12%)	14 (28%)	16 (32%)	13 (26%)
Lymph node	(3)	(1)	(4)	(6)
Bronchial hyperplasia, plasma cell	–	–	–	1 (17%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Bronchial inflammation	–	–	1 (25%)	–
Iliac, hyperplasia, lymphoid	–	–	1 (25%)	–
Lumbar, hyperplasia, lymphoid	–	–	–	1 (17%)
Mediastinal, degeneration, cystic	–	–	1 (25%)	–
Mediastinal, hemorrhage	–	1 (100%)	–	–
Mediastinal, hyperplasia	1 (33%)	–	–	–
Mediastinal, hyperplasia, plasma cell	–	–	1 (25%)	–
Mediastinal, infiltration cellular, histiocyte	–	–	–	1 (17%)
Mediastinal, pigmentation, hemosiderin	–	–	1 (25%)	1 (17%)
Renal, hyperplasia, plasma cell	–	–	–	2 (33%)
Renal, inflammation			1 (25%)	–
Lymph node, mandibular	(49)	(48)	(50)	(46)
Atrophy	–	–	1 (2%)	–
Congestion	–	–	–	1 (2%)
Degeneration, cystic	2 (4%)	4 (8%)	5 (10%)	2 (4%)
Hyperplasia, plasma cell	6 (12%)	3 (6%)	3 (6%)	4 (9%)
Inflammation	–	–	–	1 (2%)
Lymph node, mesenteric	(50)	(49)	(48)	(45)
Atrophy	–	–	–	1 (2%)
Congestion	–	–	–	1 (2%)
Hyperplasia, plasma cell	–	–	1 (2%)	1 (2%)
Infiltration cellular, histiocyte	–	–	–	1 (2%)
Spleen	(50)	(49)	(48)	(43)
Depletion lymphoid	–	7 (14%)	5 (10%)	17 (40%)
Fibrosis	–	1 (2%)	–	–
Hematopoietic cell proliferation	11 (22%)	7 (14%)	10 (21%)	5 (12%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Inflammation	–	–	1 (2%)	–
Lymphoid follicle, hyperplasia	1 (2%)	–	–	–
Thymus	(49)	(47)	(50)	(47)
Cyst	–	–	–	1 (2%)
Hemorrhage	–	–	–	1 (2%)
Inflammation	–	1 (2%)	–	–
Integumentary System				

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Mammary gland	(50)	(49)	(49)	(47)
Hyperplasia	3 (6%)	3 (6%)	1 (2%)	–
Duct, dilatation	–	–	–	1 (2%)
Epithelium, cytoplasmic alteration	–	1 (2%)	–	–
Skin	(50)	(50)	(50)	(50)
Hyperkeratosis	1 (2%)	–	–	–
Hyperplasia	–	–	–	1 (2%)
Inflammation	–	–	–	1 (2%)
Ulcer	–	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(49)
Skeletal muscle	(0)	(0)	(3)	(2)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	2 (4%)
Cerebellum, vacuolization cytoplasmic	–	1 (2%)	–	–
Peripheral nerve	(1)	(2)	(0)	(0)
Spinal cord	(1)	(2)	(0)	(1)
Degeneration	1 (100%)	–	–	–
Respiratory System				
Lung	(50)	(49)	(50)	(48)
Congestion	–	1 (2%)	2 (4%)	3 (6%)
Edema	–	2 (4%)	2 (4%)	1 (2%)
Fibrosis	–	1 (2%)	–	–
Hemorrhage	–	1 (2%)	–	2 (4%)
Inflammation, suppurative	1 (2%)	3 (6%)	2 (4%)	9 (19%)
Inflammation, chronic active	12 (24%)	11 (22%)	13 (26%)	8 (17%)
Alveolar epithelium, hyperplasia	3 (6%)	7 (14%)	7 (14%)	3 (6%)
Alveolus, infiltration cellular, histiocyte	23 (46%)	31 (63%)	18 (36%)	17 (35%)
Serosa, inflammation	–	–	1 (2%)	–
Nose	(49)	(49)	(50)	(49)
Foreign body	3 (6%)	2 (4%)	4 (8%)	8 (16%)
Inflammation, suppurative	5 (10%)	3 (6%)	17 (34%)	35 (71%)
Epithelium, goblet cell, nasopharyngeal duct, hyperplasia	4 (8%)	4 (8%)	–	–

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Epithelium, nasopharyngeal duct, accumulation, hyaline droplet	1 (2%)	5 (10%)	3 (6%)	–
Epithelium, nasopharyngeal duct, degeneration	–	–	1 (2%)	3 (6%)
Epithelium, nasopharyngeal duct, hyperplasia	–	1 (2%)	4 (8%)	1 (2%)
Epithelium, nasopharyngeal duct, inflammation, suppurative	–	–	1 (2%)	–
Epithelium, nasopharyngeal duct, necrosis	–	1 (2%)	2 (4%)	7 (14%)
Epithelium, nasopharyngeal duct, regeneration	–	–	–	8 (16%)
Lamina propria, mineralization	3 (6%)	23 (47%)	30 (60%)	22 (45%)
Lamina propria, pigmentation	1 (2%)	–	6 (12%)	14 (29%)
Nasopharyngeal duct, foreign body	1 (2%)	–	1 (2%)	2 (4%)
Nasopharyngeal duct, inflammation, suppurative	–	2 (4%)	5 (10%)	15 (31%)
Nasopharyngeal duct, mineralization	–	–	1 (2%)	–
Nerve, atrophy	–	38 (78%)	41 (82%)	38 (78%)
Olfactory epithelium, accumulation, hyaline droplet	33 (67%)	31 (63%)	17 (34%)	4 (8%)
Olfactory epithelium, atrophy	2 (4%)	35 (71%)	42 (84%)	34 (69%)
Olfactory epithelium, hyperplasia, basal cell	–	–	8 (16%)	20 (41%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)	42 (86%)	43 (86%)	36 (73%)
Olfactory epithelium, necrosis	–	3 (6%)	1 (2%)	18 (37%)
Olfactory epithelium, pigmentation	–	11 (22%)	7 (14%)	5 (10%)
Olfactory epithelium, regeneration	–	–	–	1 (2%)
Olfactory epithelium, squamous metaplasia	–	2 (4%)	1 (2%)	5 (10%)
Respiratory epithelium, accumulation, hyaline droplet	28 (57%)	31 (63%)	19 (38%)	7 (14%)
Respiratory epithelium, atrophy	–	8 (16%)	9 (18%)	3 (6%)
Respiratory epithelium, hyperplasia	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Respiratory epithelium, hyperplasia, basal cell	1 (2%)	–	–	–
Respiratory epithelium, metaplasia, squamous	1 (2%)	1 (2%)	1 (2%)	4 (8%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Respiratory epithelium, necrosis	–	1 (2%)	2 (4%)	17 (35%)
Respiratory epithelium, pigmentation	–	1 (2%)	5 (10%)	5 (10%)
Respiratory epithelium, regeneration	–	–	–	2 (4%)
Squamous epithelium, hyperplasia	–	–	–	1 (2%)
Turbinate, deformity	–	6 (12%)	20 (40%)	15 (31%)
Turbinate, hyperostosis	–	18 (37%)	32 (64%)	36 (73%)
Trachea	(50)	(50)	(50)	(50)
Hemorrhage	–	–	–	1 (2%)
Inflammation	–	–	–	1 (2%)
Special Senses System				
Eye	(48)	(50)	(50)	(49)
Cataract	–	1 (2%)	1 (2%)	–
Synechia	–	–	–	2 (4%)
Anterior chamber, hemorrhage	1 (2%)	–	–	–
Anterior chamber, posterior chamber, exudate	–	–	1 (2%)	–
Cornea, inflammation	1 (2%)	–	1 (2%)	–
Optic nerve, degeneration	1 (2%)	–	–	–
Posterior chamber, hemorrhage	–	–	1 (2%)	–
Retina, atrophy	–	1 (2%)	–	–
Retina, dysplasia	–	–	1 (2%)	1 (2%)
Harderian gland	(48)	(50)	(50)	(49)
Hyperplasia	–	–	1 (2%)	–
Inflammation	2 (4%)	3 (6%)	–	–
Zymbal's gland	(0)	(0)	(1)	(0)
Urinary System				
Kidney	(50)	(49)	(49)	(45)
Atrophy	–	–	1 (2%)	–
Cyst	1 (2%)	1 (2%)	2 (4%)	–
Hydronephrosis	2 (4%)	–	2 (4%)	3 (7%)
Infarct	–	1 (2%)	–	–
Inflammation	11 (22%)	7 (14%)	5 (10%)	1 (2%)
Necrosis	1 (2%)	–	–	–
Nephropathy	21 (42%)	16 (33%)	17 (35%)	4 (9%)
Papilla, mineralization	–	–	–	1 (2%)

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Papilla, necrosis	1 (2%)	–	1 (2%)	1 (2%)
Transitional epithelium, hyperplasia	7 (14%)	3 (6%)	6 (12%)	–
Urinary bladder	(49)	(49)	(49)	(44)
Inflammation	1 (2%)	1 (2%)	–	–

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

Table B-4. Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus in Female Rats in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	100 mg/kg	300 mg/kg	1,000 mg/kg
Original Evaluation				
Number microscopically examined	50	49	49	44
Adenoma[a]	0	0	0	1 (2%)
Adenocarcinoma	3 (6%)	9 (18%)	5 (10%)	3 (7%)
Adenocarcinoma, squamous differentiation	0	0	0	1 (2%)
Adenocarcinoma (includes squamous differentiation)[b]				
Overall rate[c]	3/50 (6%)	9/50 (18%)	5/50 (10%)	4/50 (8%)
Adjusted rate[d]	7.3%	21.9%	14.9%	23.0%
Terminal rate[e]	1/26 (4%)	4/28 (14%)	2/23 (9%)	1/4 (25%)
First incidence (days)	688	484	610	533
Poly-3 test[f]	P = 0.202	P = 0.056	P = 0.248	P = 0.141
Residual Tissue Evaluation				
Number microscopically examined	44	49	49	44
Endometrium, hyperplasia, atypical	2 (5%)	4 (8%)	4 (8%)	3 (7%)
Adenoma	0	1 (2%)	1 (2%)	2 (5%)
Adenocarcinoma	7 (16%)	12 (24%)	10 (20%)	7 (16%)
Adenocarcinoma, squamous differentiation	0	0	0	1 (2%)
Original and Residual Tissue Evaluations (Combined)				
Number microscopically examined	44	49	49	44
Endometrium, hyperplasia, atypical	2 (5%)	4 (8%)	4 (8%)	3 (7%)
Adenoma	0	1 (2%)	1 (2%)	2 (5%)
Adenocarcinoma	8 (18%)	13 (27%)	10 (20%)	7 (16%)
Adenocarcinoma, squamous differentiation	0	0	0	1 (2%)

[a]Number of animals with neoplasm.
[b]Historical control incidence for 2-year studies in Wistar Han rats (all routes): 7/150 (includes one endometrium carcinoma).
[c]Number of animals with neoplasm per number of animals necropsied.
[d]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[e]Observed incidence at terminal kill.
[f]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.

Appendix C. Summary of Lesions in Male Mice in the Two-year Gavage Study of Green Tea Extract

Tables

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Gavage Study of Green Tea Extract[a]

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	–	–	–	2
Moribund	6	8	7	10
Natural deaths	11	6	10	1
Survivors				
Died last week of study	–	–	1	–
Terminal kill	33	36	32	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(49)	(50)	(50)
Gallbladder	(50)	(48)	(49)	(49)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Leiomyoma	–	–	1 (2%)	–
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(43)	(50)	(47)	(49)
Adenoma	–	–	1 (2%)	–
Intestine small, ileum	(47)	(50)	(48)	(50)
Intestine small, jejunum	(45)	(50)	(49)	(50)
Adenocarcinoma	–	–	1 (2%)	1 (2%)
Adenoma	–	–	–	1 (2%)
Carcinoma	2 (4%)	1 (2%)	–	–
Liver	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	–
Hemangioma	2 (4%)	–	2 (4%)	–
Hemangiosarcoma	3 (6%)	3 (6%)	3 (6%)	–
Hepatoblastoma	2 (4%)	–	–	1 (2%)
Hepatocellular adenoma	19 (38%)	14 (28%)	19 (38%)	14 (28%)
Hepatocellular adenoma, multiple	16 (32%)	15 (30%)	6 (12%)	2 (4%)
Hepatocellular carcinoma	13 (26%)	11 (22%)	8 (16%)	7 (14%)
Hepatocellular carcinoma, multiple	2 (4%)	7 (14%)	5 (10%)	1 (2%)
Hepatocholangiocarcinoma	–	3 (6%)	–	–

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Squamous cell carcinoma, metastatic, stomach, forestomach	–	1 (2%)	–	–
Mesentery	(1)	(4)	(2)	(1)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma	–	1 (2%)	–	–
Squamous cell papilloma	–	–	–	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Tooth	(0)	(1)	(0)	(1)
Odontoma	–	1 (100%)	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, hepatoblastoma, metastatic, liver	–	–	–	1 (2%)
Heart	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver	–	–	–	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver	–	1 (2%)	–	–
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Adenoma	–	–	1 (2%)	–
Hepatoblastoma, metastatic, liver	–	–	–	1 (2%)
Subcapsular, adenoma	1 (2%)	–	1 (2%)	–
Adrenal medulla	(49)	(50)	(50)	(50)
Pheochromocytoma benign	–	1 (2%)	1 (2%)	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	3 (6%)	–	–
Carcinoma	1 (2%)	–	–	–
Parathyroid gland	(41)	(30)	(46)	(44)
Pituitary gland	(49)	(50)	(49)	(50)
Pars distalis, adenoma	1 (2%)	–	–	–
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, carcinoma	–	–	–	1 (2%)
Follicular cell, adenoma	1 (2%)	2 (4%)	–	–

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
General Body System				
None				
Genital System				
Coagulating gland	(1)	(0)	(0)	(0)
Sarcoma	1 (100%)	–	–	–
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	1 (2%)	3 (6%)	–	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	–	–	–
Lymph node	(2)	(3)	(1)	(0)
Renal, hemangiosarcoma	–	–	1 (100%)	–
Lymph node, mandibular	(50)	(50)	(50)	(50)
Lymph node, mesenteric	(50)	(49)	(48)	(50)
Hemangiosarcoma	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	1 (2%)	–	–
Thymus	(44)	(49)	(46)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	–
Integumentary System				
Mammary gland	(0)	(2)	(0)	(1)
Skin	(50)	(50)	(50)	(50)
Carcinoma, metastatic, liver	1 (2%)	–	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(1)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver	–	1 (2%)	–	–

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	12 (24%)	7 (14%)	8 (16%)	4 (8%)
Alveolar/bronchiolar adenoma, multiple	–	1 (2%)	2 (4%)	1 (2%)
Alveolar/bronchiolar carcinoma	–	3 (6%)	2 (4%)	–
Alveolar/bronchiolar carcinoma, multiple	2 (4%)	4 (8%)	1 (2%)	–
Carcinoma, metastatic, Harderian gland	–	1 (2%)	–	–
Carcinoma, metastatic, kidney	1 (2%)	–	–	–
Hepatoblastoma, metastatic, liver				1 (2%)
Hepatocellular carcinoma, metastatic, liver	4 (8%)	5 (10%)	2 (4%)	–
Hepatocholangiocarcinoma, metastatic, liver	–	3 (6%)	–	–
Mediastinum, hepatocellular carcinoma, metastatic, liver	1 (2%)	–	–	–
Nose	(50)	(49)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	7 (14%)	1 (2%)	6 (12%)	4 (8%)
Carcinoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hemangioma	–	–	–	1 (2%)
Hepatoblastoma, metastatic, liver	–	–	–	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver	–	1 (2%)	–	–
Bilateral, renal tubule, carcinoma	2 (4%)	–	–	–
Renal tubule, adenoma	1 (2%)	–	–	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	1 (2%)	–	–
Lymphoma malignant	4 (8%)	6 (12%)	2 (4%)	–
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	48	42	30
Total primary neoplasms	99	91	72	42
Total animals with benign neoplasms	43	37	31	26
Total benign neoplasms	62	49	48	30
Total animals with malignant neoplasms	28	34	23	12
Total malignant neoplasms	37	42	24	12
Total animals with metastatic neoplasms	5	12	2	1
Total metastatic neoplasms	7	15	2	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 C-2. Statistical Analysis of Primary Neoplasms in Male Mice in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	7/50 (14%)	1/50 (2%)	6/50 (12%)	4/50 (8%)
Adjusted rate[b]	15.7%	2.2%	13.1%	8.8%
Terminal rate[c]	6/33 (18%)	0/36 (0%)	4/32 (13%)	3/37 (8%)
First incidence (days)	616	708	685	675
Poly-3 test[d]	P = 0.495N	P = 0.028N	P = 0.479N	P = 0.252N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	2/50 (4%)	7/50 (14%)	5/50 (10%)
Adjusted rate	17.9%	4.4%	15.3%	11.0%
Terminal rate	7/33 (21%)	1/36 (3%)	5/32 (16%)	4/37 (11%)
First incidence (days)	616	708	685	675
Poly-3 test	P = 0.495N	P = 0.042N	P = 0.477N	P = 0.265N
Kidney (Renal Tubule): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.7%	0.0%	0.0%	2.2%
Terminal rate	1/33 (3%)	0/36 (0%)	0/32 (0%)	1/37 (3%)
First incidence (days)	637	–[e]	–	730 (T)
Poly-3 test	P = 0.451N	P = 0.116N	P = 0.116N	P = 0.303N
Liver: Hemangiosarcoma				
Overall rate	3/50 (6%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	6.8%	6.6%	6.6%	0.0%
Terminal rate	3/33 (9%)	3/36 (8%)	2/32 (6%)	0/37 (0%)
First incidence (days)	730 (T)	730 (T)	675	–
Poly-3 test	P = 0.080N	P = 0.651N	P = 0.647N	P = 0.115N
Liver: Hepatocellular Adenoma				
Overall rate	35/50 (70%)	29/50 (58%)	25/50 (50%)	16/50 (32%)
Adjusted rate	74.6%	61.5%	52.7%	34.8%
Terminal rate	26/33 (79%)	24/36 (67%)	15/32 (47%)	14/37 (38%)
First incidence (days)	587	528	536	450
Poly-3 test	P < 0.001N	P = 0.119N	P = 0.019N	P < 0.001N
Liver: Hepatocellular Carcinoma				
Overall rate	15/50 (30%)	18/50 (36%)	13/50 (26%)	8/50 (16%)
Adjusted rate	32.4%	38.4%	27.4%	17.5%
Terminal rate	10/33 (30%)	12/36 (33%)	5/32 (16%)	6/37 (16%)
First incidence (days)	335	462	514	592
Poly-3 test	P = 0.023N	P = 0.350	P = 0.379N	P = 0.076N

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	40/50 (80%)	39/50 (78%)	35/50 (70%)	21/50 (42%)
Adjusted rate	82.6%	80.0%	71.1%	44.9%
Terminal rate	28/33 (85%)	28/36 (78%)	19/32 (59%)	17/37 (46%)
First incidence (days)	335	462	514	450
Poly-3 test	P < 0.001N	P = 0.475N	P = 0.128N	P < 0.001N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	17/50 (34%)	18/50 (36%)	13/50 (26%)	9/50 (18%)
Adjusted rate	36.4%	38.4%	27.4%	19.5%
Terminal rate	11/33 (33%)	12/36 (33%)	5/32 (16%)	6/37 (16%)
First incidence (days)	335	462	514	592
Poly-3 test	P = 0.023N	P = 0.506	P = 0.236N	P = 0.054N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	40/50 (80%)	39/50 (78%)	35/50 (70%)	22/50 (44%)
Adjusted rate	82.6%	80.0%	71.1%	46.7%
Terminal rate	28/33 (85%)	28/36 (78%)	19/32 (59%)	17/37 (46%)
First incidence (days)	335	462	514	450
Poly-3 test	P < 0.001N	P = 0.475N	P = 0.128N	P < 0.001N
Liver: Hepatocholangiocarcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	6.5%	0.0%	0.0%
Terminal rate	0/33 (0%)	1/36 (3%)	0/32 (0%)	0/37 (0%)
First incidence (days)	–	546	–	–
Poly-3 test	P = 0.242N	P = 0.127	–[f]	–
Lung: Alveolar/Bronchiolar Adenoma				
Overall rate	12/50 (24%)	8/50 (16%)	10/50 (20%)	5/50 (10%)
Adjusted rate	26.6%	17.5%	21.7%	10.9%
Terminal rate	8/33 (24%)	6/36 (17%)	7/32 (22%)	4/37 (11%)
First incidence (days)	587	644	632	449
Poly-3 test	P = 0.065N	P = 0.213N	P = 0.379N	P = 0.047N
Lung: Alveolar/Bronchiolar Carcinoma				
Overall rate	2/50 (4%)	7/50 (14%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.5%	15.2%	6.6%	0.0%
Terminal rate	1/33 (3%)	4/36 (11%)	3/32 (9%)	0/37 (0%)
First incidence (days)	721	637	730 (T)	–
Poly-3 test	P = 0.037N	P = 0.087	P = 0.513	P = 0.233N

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma				
Overall rate	14/50 (28%)	15/50 (30%)	12/50 (24%)	5/50 (10%)
Adjusted rate	31.0%	32.4%	26.0%	10.9%
Terminal rate	9/33 (27%)	10/36 (28%)	9/32 (28%)	4/37 (11%)
First incidence (days)	587	637	632	449
Poly-3 test	P = 0.006N	P = 0.534	P = 0.382N	P = 0.016N
Pancreatic Islets: Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.2%	6.6%	0.0%	0.0%
Terminal rate	0/33 (0%)	2/36 (6%)	0/32 (0%)	0/37 (0%)
First incidence (days)	616	658	–	–
Poly-3 test	P = 0.146N	P = 0.313	P = 0.496N	P = 0.498N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	4.5%	6.6%	0.0%	0.0%
Terminal rate	1/33 (3%)	2/36 (6%)	0/32 (0%)	0/37 (0%)
First incidence (days)	616	658	–	–
Poly-3 test	P = 0.087N	P = 0.510	P = 0.233N	P = 0.235N
Testes: Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.2%	6.6%	0.0%	2.2%
Terminal rate	0/33 (0%)	3/36 (8%)	0/32 (0%)	1/37 (3%)
First incidence (days)	553	730 (T)	–	730 (T)
Poly-3 test	P = 0.425N	P = 0.309	P = 0.497N	P = 0.759N
All Organs: Hemangiosarcoma				
Overall rate	5/50 (10%)	4/50 (8%)	4/50 (8%)	0/50 (0%)
Adjusted rate	11.3%	8.8%	8.7%	0.0%
Terminal rate	5/33 (15%)	4/36 (11%)	3/32 (9%)	0/37 (0%)
First incidence (days)	730 (T)	730 (T)	675	–
Poly-3 test	P = 0.027N	P = 0.484N	P = 0.479N	P = 0.029N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	7/50 (14%)	4/50 (8%)	6/50 (12%)	1/50 (2%)
Adjusted rate	15.7%	8.8%	13.1%	2.2%
Terminal rate	5/33 (15%)	4/36 (11%)	5/32 (16%)	0/37 (0%)
First incidence (days)	668	730 (T)	675	693
Poly-3 test	P = 0.040N	P = 0.250N	P = 0.479N	P = 0.028N

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
All Organs: Malignant Lymphoma				
Overall rate	4/50 (8%)	6/50 (12%)	2/50 (4%)	0/50 (0%)
Adjusted rate	9.0%	13.0%	4.4%	0.0%
Terminal rate	4/33 (12%)	4/36 (11%)	2/32 (6%)	0/37 (0%)
First incidence (days)	730 (T)	528	730 (T)	–
Poly-3 test	P = 0.018N	P = 0.394	P = 0.324N	P = 0.058N
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	37/50 (74%)	31/50 (62%)	26/50 (52%)
Adjusted rate	89.4%	77.6%	64.9%	55.2%
Terminal rate	31/33 (94%)	28/36 (78%)	20/32 (63%)	21/37 (57%)
First incidence (days)	553	528	536	449
Poly-3 test	P < 0.001N	P = 0.089N	P = 0.002N	P < 0.001N
All Organs: Malignant Neoplasms				
Overall rate	28/50 (56%)	34/50 (68%)	23/50 (46%)	12/50 (24%)
Adjusted rate	58.7%	69.4%	48.0%	26.0%
Terminal rate	18/33 (55%)	23/36 (64%)	13/32 (41%)	9/37 (24%)
First incidence (days)	335	462	514	592
Poly-3 test	P < 0.001N	P = 0.184	P = 0.198N	P < 0.001N
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	48/50 (96%)	42/50 (84%)	30/50 (60%)
Adjusted rate	98.0%	96.0%	84.7%	62.3%
Terminal rate	32/33 (97%)	34/36 (94%)	25/32 (78%)	22/37 (60%)
First incidence (days)	335	462	514	449
Poly-3 test	P < 0.001N	P = 0.500N	P = 0.019N	P < 0.001N

T = terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for kidney, liver, lung, pancreatic islets, and testes; 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 C-3. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Two-year Gavage Study of Green Tea Extract[a]

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	–	–	–	2
Moribund	6	8	7	10
Natural deaths	11	6	10	1
Survivors				
Died last week of study	–	–	1	–
Terminal kill	33	36	32	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(49)	(50)	(50)
Inflammation	–	1 (2%)	1 (2%)	1 (2%)
Perforation	–	–	–	1 (2%)
Muscularis, degeneration	1 (2%)	–	–	–
Periesophageal tissue, inflammation	–	–	1 (2%)	–
Gallbladder	(50)	(48)	(49)	(49)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation	–	1 (2%)	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Epithelium, hyperplasia, adenomatous	–	–	–	1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(43)	(50)	(47)	(49)
Intestine small, ileum	(47)	(50)	(48)	(50)
Hyperplasia	–	1 (2%)	–	–
Inflammation	–	1 (2%)	–	–
Intestine small, jejunum	(45)	(50)	(49)	(50)
Angiectasis	–	–	–	1 (2%)
Inflammation, granulomatous	–	–	1 (2%)	–
Peyer's patch, hyperplasia, lymphoid	1 (2%)	–	–	–
Liver	(50)	(50)	(50)	(50)
Basophilic focus	8 (16%)	13 (26%)	6 (12%)	6 (12%)
Clear cell focus	24 (48%)	17 (34%)	6 (12%)	1 (2%)

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Embolus bacterial	–	–	3 (6%)	–
Eosinophilic focus	24 (48%)	27 (54%)	22 (44%)	13 (26%)
Fatty change	2 (4%)	1 (2%)	–	1 (2%)
Fibrosis	–	1 (2%)	–	–
Hematopoietic cell proliferation	2 (4%)	2 (4%)	6 (12%)	10 (20%)
Hemorrhage	1 (2%)	–	–	
Hepatodiaphragmatic nodule	–	2 (4%)	–	1 (2%)
Infarct	–	1 (2%)	–	–
Infiltration cellular, lymphocyte	–	1 (2%)	1 (2%)	1 (2%)
Inflammation	4 (8%)	1 (2%)	5 (10%)	12 (24%)
Mixed cell focus	9 (18%)	4 (8%)	5 (10%)	2 (4%)
Tension lipidosis	–	–	1 (2%)	–
Thrombosis	–	2 (4%)	–	–
Bile duct, cyst	1 (2%)	–	–	–
Hepatocyte, necrosis	7 (14%)	12 (24%)	7 (14%)	4 (8%)
Hepatocyte, vacuolization cytoplasmic	–	–	1 (2%)	–
Kupffer cell, hyperplasia	3 (6%)	–	–	–
Mesentery	(1)	(4)	(2)	(1)
Inflammation	–	–	2 (100%)	–
Fat, necrosis	1 (100%)	4 (100%)	–	1 (100%)
Pancreas	(50)	(50)	(50)	(50)
Acinus, atrophy	–	–	–	4 (8%)
Duct, inflammation	–	–	1 (2%)	–
Salivary glands	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Necrosis	1 (2%)	–	–	–
Duct, hyperplasia	–	1 (2%)	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation	5 (10%)	3 (6%)	1 (2%)	2 (4%)
Ulcer	3 (6%)	–	2 (4%)	–
Epithelium, hyperplasia	5 (10%)	4 (8%)	2 (4%)	1 (2%)
Epithelium, hyperplasia, focal	1 (2%)	–	–	–
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	2 (4%)	–	1 (2%)	–
Fungus	–	–	1 (2%)	–

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Inflammation	1 (2%)	2 (4%)	1 (2%)	–
Metaplasia, squamous	–	1 (2%)	–	–
Epithelium, mineralization	–	–	1 (2%)	–
Epithelium, necrosis	–	–	1 (2%)	–
Glands, hyperplasia	2 (4%)	–	–	–
Tooth	(0)	(1)	(0)	(1)
Inflammation, chronic active	–	–	–	1 (100%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, inflammation	–	–	2 (4%)	1 (2%)
Carotid artery, inflammation	–	–	–	1 (2%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	15 (30%)	15 (30%)	11 (22%)	11 (22%)
Inflammation	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Mineralization	–	–	1 (2%)	–
Thrombosis	–	–	1 (2%)	–
Artery, inflammation	1 (2%)	–	2 (4%)	2 (4%)
Pericardium, inflammation	–	–	1 (2%)	–
Valve, thrombosis	3 (6%)	1 (2%)	–	–
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Atrophy	–	–	1 (2%)	1 (2%)
Degeneration, cystic	–	1 (2%)	–	–
Hyperplasia	2 (4%)	3 (6%)	–	3 (6%)
Hypertrophy	10 (20%)	8 (16%)	1 (2%)	2 (4%)
Necrosis	–	–	1 (2%)	–
Adrenal medulla	(49)	(50)	(50)	(50)
Hyperplasia	–	1 (2%)	1 (2%)	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	22 (44%)	18 (36%)	19 (38%)	1 (2%)
Parathyroid gland	(41)	(30)	(46)	(44)
Pituitary gland	(49)	(50)	(49)	(50)
Pars distalis, hyperplasia	2 (4%)	–	–	–
Thyroid gland	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	–

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
General Body System				
None	–	–	–	–
Genital System				
Coagulating gland	(1)	(0)	(0)	(0)
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)	–	–	–
Preputial gland	(50)	(50)	(50)	(50)
Ectasia	1 (2%)	4 (8%)	5 (10%)	4 (8%)
Inflammation	–	1 (2%)	–	–
Prostate	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)	–	–
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation	–	–	–	1 (2%)
Infiltration cellular, lymphocyte	–	1 (2%)	–	–
Inflammation	1 (2%)	1 (2%)	–	1 (2%)
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, degeneration	4 (8%)	3 (6%)	1 (2%)	–
Germinal epithelium, mineralization	1 (2%)	–	–	–
Interstitial cell, hyperplasia	2 (4%)	1 (2%)	–	2 (4%)
Tunic, inflammation	–	–	1 (2%)	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	42 (84%)	38 (76%)	46 (92%)
Infiltration cellular, plasma cell	1 (2%)	–	–	–
Lymph node	(2)	(3)	(1)	(0)
Infiltration cellular, plasma cell	–	1 (33%)	–	–
Lymph node, mandibular	(50)	(50)	(50)	(50)
Atrophy	–	–	1 (2%)	1 (2%)
Hyperplasia, lymphoid	–	1 (2%)	31 (62%)	37 (74%)
Infiltration cellular, plasma cell	1 (2%)	1 (2%)	24 (48%)	41 (82%)
Inflammation	–	–	1 (2%)	–
Necrosis	–	–	1 (2%)	–
Lymph node, mesenteric	(50)	(49)	(48)	(50)
Amyloid deposition	–	1 (2%)	–	–
Atrophy	3 (6%)	2 (4%)	1 (2%)	7 (14%)

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Ectasia	1 (2%)	1 (2%)	–	–
Fibrosis	–	1 (2%)	–	–
Hematopoietic cell proliferation	1 (2%)	–	–	–
Hyperplasia, lymphoid	3 (6%)	3 (6%)	1 (2%)	1 (2%)
Inflammation	2 (4%)	1 (2%)	1 (2%)	–
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	18 (36%)	25 (50%)	29 (58%)	25 (50%)
Infiltration cellular, plasma cell	–	1 (2%)	–	–
Pigmentation, hemosiderin	–	–	–	2 (4%)
Lymphoid follicle, hyperplasia	9 (18%)	14 (28%)	7 (14%)	–
Red pulp, atrophy	2 (4%)	3 (6%)	1 (2%)	1 (2%)
White pulp, atrophy	9 (18%)	7 (14%)	13 (26%)	2 (4%)
Thymus	(44)	(49)	(46)	(48)
Atrophy	44 (100%)	45 (92%)	45 (98%)	44 (92%)
Integumentary System				
Mammary gland	(0)	(2)	(0)	(1)
Skin	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	1 (2%)	1 (2%)	–
Ulcer	1 (2%)	–	–	3 (6%)
Subcutaneous tissue, necrosis	–	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Fibrous dysplasia	–	–	–	1 (2%)
Tendon, inflammation	1 (2%)	–	–	–
Skeletal muscle	(0)	(1)	(1)	(0)
Inflammation	–	1 (100%)	1 (100%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	–	1 (2%)	–	–
Edema, multifocal	–	–	1 (2%)	–
Hemorrhage	–	–	1 (2%)	–
Inflammation	–	–	2 (4%)	–
Necrosis	–	1 (2%)	1 (2%)	–

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	–	–	1 (2%)	–
Infiltration cellular, lymphocyte	1 (2%)	–	–	4 (8%)
Inflammation	1 (2%)	–	6 (12%)	5 (10%)
Thrombosis	1 (2%)	1 (2%)	1 (2%)	–
Alveolar epithelium, hyperplasia	5 (10%)	4 (8%)	5 (10%)	1 (2%)
Alveolus, infiltration cellular, histiocyte	2 (4%)	1 (2%)	–	–
Bronchiole, hyperplasia	1 (2%)	1 (2%)	–	–
Mediastinum, inflammation	–	–	2 (4%)	–
Mediastinum, necrosis	–	–	1 (2%)	–
Nose	(50)	(49)	(50)	(50)
Foreign body	1 (2%)	10 (20%)	16 (32%)	25 (50%)
Hemorrhage	–	–	1 (2%)	–
Hyperostosis	–	–	28 (56%)	46 (92%)
Inflammation, suppurative	14 (28%)	40 (82%)	49 (98%)	48 (96%)
Polyp, inflammatory	3 (6%)	–	–	–
Glands, respiratory epithelium, hyperplasia	3 (6%)	8 (16%)	3 (6%)	1 (2%)
Lumen, pigmentation	–	3 (6%)	4 (8%)	2 (4%)
Nasopharyngeal duct, degeneration	–	–	4 (8%)	9 (18%)
Nasopharyngeal duct, inflammation	–	1 (2%)	2 (4%)	4 (8%)
Nasopharyngeal duct, metaplasia, squamous	–	–	–	1 (2%)
Nerve, atrophy	–	26 (53%)	49 (98%)	50 (100%)
Olfactory epithelium, accumulation, hyaline droplet	37 (74%)	47 (96%)	3 (6%)	–
Olfactory epithelium, atrophy	4 (8%)	24 (49%)	28 (56%)	3 (6%)
Olfactory epithelium, fibrosis	–	4 (8%)	37 (74%)	43 (86%)
Olfactory epithelium, metaplasia, respiratory	11 (22%)	45 (92%)	49 (98%)	49 (98%)
Olfactory epithelium, metaplasia, squamous	1 (2%)	–	1 (2%)	–
Olfactory epithelium, necrosis	–	2 (4%)	1 (2%)	–
Respiratory epithelium, accumulation, hyaline droplet	49 (98%)	49 (100%)	34 (68%)	5 (10%)
Respiratory epithelium, hyperplasia	5 (10%)	20 (41%)	10 (20%)	19 (38%)

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Respiratory epithelium, metaplasia, squamous	–	14 (29%)	39 (78%)	46 (92%)
Respiratory epithelium, necrosis	–	7 (14%)	16 (32%)	27 (54%)
Septum, perforation	1 (2%)	–	26 (52%)	37 (74%)
Squamous epithelium, necrosis	–	1 (2%)	–	–
Turbinate, atrophy	–	–	41 (82%)	50 (100%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cornea, inflammation	1 (2%)	–	–	–
Optic nerve, demyelination	–	–	–	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	1 (2%)	4 (8%)	1 (2%)
Inflammation	–	–	1 (2%)	–
Necrosis	–	–	1 (2%)	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	–	1 (2%)	–	–
Cyst	–	1 (2%)	–	–
Hydronephrosis	–	–	–	1 (2%)
Infarct	5 (10%)	3 (6%)	7 (14%)	4 (8%)
Infiltration cellular, lymphocyte	1 (2%)	–	1 (2%)	–
Infiltration cellular, plasma cell	–	1 (2%)	–	–
Inflammation	–	–	2 (4%)	–
Nephropathy	38 (76%)	41 (82%)	35 (70%)	42 (84%)
Artery, inflammation	–	–	–	1 (2%)
Cortex, cyst	3 (6%)	1 (2%)	2 (4%)	2 (4%)
Pelvis, inflammation	–	2 (4%)	–	–
Renal tubule, hyperplasia	2 (4%)	2 (4%)	–	–
Renal tubule, necrosis	–	2 (4%)	–	–
Vein, inflammation, chronic active	–	–	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Calculus gross observation	–	1 (2%)	–	–

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

Appendix D. Summary of Lesions in Female Mice in the Two-year Gavage Study of Green Tea Extract

Tables

Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Gavage Study of Green Tea Extract[a]

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	3	6	1	–
Moribund	10	2	2	6
Natural deaths	3	9	3	5
Survivors				
Died last week of study	–	1	–	–
Terminal kill	34	32	44	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(49)	(49)	(50)
Gallbladder	(49)	(48)	(50)	(49)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(48)	(40)	(49)	(48)
Adenoma	–	1 (3%)	–	–
Epithelium, adenocarcinoma	–	–	1 (2%)	–
Intestine small, ileum	(49)	(46)	(49)	(48)
Intestine small, jejunum	(48)	(43)	(49)	(48)
Leiomyosarcoma	1 (2%)	–	–	–
Liver	(50)	(50)	(50)	(50)
Cystadenocarcinoma, metastatic, ovary	1 (2%)	–	–	–
Hemangiosarcoma	1 (2%)	–	–	–
Hepatocellular adenoma	7 (14%)	8 (16%)	3 (6%)	–
Hepatocellular adenoma, multiple	5 (10%)	1 (2%)	–	–
Hepatocellular carcinoma	5 (10%)	6 (12%)	4 (8%)	1 (2%)
Hepatocellular carcinoma, multiple	–	1 (2%)	–	–
Hepatocholangiocarcinoma, multiple	–	–	1 (2%)	–
Mesentery	(6)	(0)	(3)	(2)
Hemangiosarcoma	–	–	1 (33%)	–
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (17%)	–	–	–

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Pancreas	(50)	(49)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Salivary glands	(50)	(48)	(49)	(48)
Stomach, forestomach	(49)	(49)	(50)	(49)
Squamous cell carcinoma	1 (2%)	–	–	–
Squamous cell papilloma	1 (2%)	–	–	–
Stomach, glandular	(49)	(48)	(49)	(49)
Tongue	(0)	(0)	(0)	(2)
Squamous cell carcinoma	–	–	–	1 (50%)
Squamous cell papilloma	–	–	–	1 (50%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(49)	(50)	(50)
Endocrine system				
Adrenal cortex	(49)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver	–	1 (2%)	–	–
Subcapsular, adenoma	–	1 (2%)	–	–
Adrenal medulla	(49)	(50)	(50)	(50)
Pheochromocytoma benign	–	1 (2%)	1 (2%)	–
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma	1 (2%)	–	–	1 (2%)
Parathyroid gland	(35)	(40)	(41)	(35)
Pituitary gland	(50)	(48)	(49)	(50)
Thyroid gland	(50)	(48)	(49)	(49)
C-cell, carcinoma	–	1 (2%)	–	–
Follicular cell, adenoma	–	1 (2%)	–	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(49)	(49)	(50)	(50)
Ovary	(50)	(50)	(50)	(49)
Cystadenocarcinoma	1 (2%)	1 (2%)	–	–
Cystadenoma	2 (4%)	1 (2%)	5 (10%)	4 (8%)
Sarcoma	1 (2%)	–	–	–

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Bilateral, cystadenoma	1 (2%)	–	–	–
Uterus	(50)	(50)	(50)	(50)
Polyp stromal	2 (4%)	–	3 (6%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiocarcinoma	1 (2%)	–	–	–
Lymph node	(5)	(5)	(3)	(2)
Lymph node, mandibular	(50)	(48)	(49)	(48)
Lymph node, mesenteric	(50)	(49)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Spleen	(50)	(49)	(50)	(50)
Thymus	(47)	(49)	(49)	(50)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Skin	(50)	(50)	(50)	(50)
Fibrosarcoma	–	–	–	1 (2%)
Subcutaneous tissue, fibrosarcoma	–	1 (2%)	–	–
Subcutaneous tissue, hemangiosarcoma	–	–	1 (2%)	–
Subcutaneous tissue, sarcoma	–	–	–	1 (2%)
Subcutaneous tissue, schwannoma malignant	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(4)	(0)	(0)	(3)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (25%)	–	–	–
Rhabdomyosarcoma	2 (50%)	–	–	2 (67%)
Nervous System				
Brain	(50)	(49)	(50)	(50)
Respiratory System				
Lung	(50)	(49)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	2 (4%)	1 (2%)	3 (6%)
Alveolar/bronchiolar carcinoma	–	1 (2%)	1 (2%)	1 (2%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	–	–	–

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Carcinoma, metastatic, Harderian gland	1 (2%)	–	–	–
Hepatocellular carcinoma, metastatic, liver	1 (2%)	3 (6%)	–	–
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)	–	–	–
Mediastinum, rhabdomyosarcoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Nose	(48)	(48)	(50)	(50)
Glands, adenoma	1 (2%)	–	–	–
Trachea	(50)	(48)	(49)	(49)
Special Senses System				
Eye	(50)	(48)	(49)	(50)
Harderian gland	(50)	(46)	(49)	(50)
Adenoma	2 (4%)	1 (2%)	3 (6%)	3 (6%)
Carcinoma	2 (4%)	–	–	1 (2%)
Zymbal's gland	(0)	(0)	(0)	(1)
Carcinoma	–	–	–	1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Bilateral, renal tubule, carcinoma	–	–	1 (2%)	–
Renal tubule, carcinoma	–	–	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)	–	–	–
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	2 (4%)	1 (2%)	1 (2%)
Lymphoma malignant	7 (14%)	9 (18%)	6 (12%)	4 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	29	30	23	22
Total primary neoplasms	50	40	34	27
Total animals with benign neoplasms	20	17	13	13
Total benign neoplasms	27	17	16	13
Total animals with malignant neoplasms	18	18	16	12
Total malignant neoplasms	23	23	18	14

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Total animals with metastatic neoplasms	5	3	1	1
Total metastatic neoplasms	7	4	3	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 D-2. Statistical Analysis of Primary Neoplasms in Female Mice in the Two-year Gavage Study of Green Tea Extract

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	2/50 (4%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate[b]	4.6%	2.5%	6.3%	6.7%
Terminal rate[c]	1/34 (3%)	1/33 (3%)	2/44 (5%)	3/39 (8%)
First incidence (days)	722	729 (T)	564	729 (T)
Poly-3 test[d]	P = 0.350	P = 0.528N	P = 0.538	P = 0.510
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	3/50 (6%)	4/50 (8%)
Adjusted rate	9.1%	2.5%	6.3%	8.9%
Terminal rate	3/34 (9%)	1/33 (3%)	2/44 (5%)	4/39 (10%)
First incidence (days)	722	729 (T)	564	729 (T)
Poly-3 test	P = 0.399	P = 0.204N	P = 0.455N	P = 0.631N
Liver: Hepatocellular Adenoma				
Overall rate	12/50 (24%)	9/50 (18%)	3/50 (6%)	0/50 (0%)
Adjusted rate	27.2%	22.3%	6.4%	0.0%
Terminal rate	10/34 (29%)	9/33 (27%)	3/44 (7%)	0/39 (0%)
First incidence (days)	661	729 (T)	729 (T)	–[e]
Poly-3 test	P < 0.001N	P = 0.393N	P = 0.007N	P < 0.001N
Liver: Hepatocellular Carcinoma				
Overall rate	5/50 (10%)	7/50 (14%)	4/50 (8%)	1/50 (2%)
Adjusted rate	11.4%	17.0%	8.5%	2.2%
Terminal rate	3/34 (9%)	3/33 (9%)	4/44 (9%)	0/39 (0%)
First incidence (days)	709	630	729 (T)	715
Poly-3 test	P = 0.032N	P = 0.333	P = 0.457N	P = 0.096N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	14/50 (28%)	15/50 (30%)	7/50 (14%)	1/50 (2%)
Adjusted rate	31.7%	36.5%	14.9%	2.2%
Terminal rate	11/34 (32%)	11/33 (33%)	7/44 (16%)	0/39 (0%)
First incidence (days)	661	630	729 (T)	715
Poly-3 test	P < 0.001N	P = 0.407	P = 0.047N	P < 0.001N
Lung: Alveolar/Bronchiolar Adenoma				
Overall rate	5/50 (10%)	2/49 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	11.4%	5.1%	2.1%	6.7%
Terminal rate	4/34 (12%)	2/33 (6%)	1/44 (2%)	3/39 (8%)

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
First incidence (days)	722	729 (T)	729 (T)	729 (T)
Poly-3 test	P = 0.446N	P = 0.258N	P = 0.086N	P = 0.344N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma				
Overall rate	6/50 (12%)	3/49 (6%)	2/50 (4%)	4/50 (8%)
Adjusted rate	13.7%	7.6%	4.3%	8.9%
Terminal rate	4/34 (12%)	3/33 (9%)	2/44 (5%)	4/39 (10%)
First incidence (days)	686	729 (T)	729 (T)	729 (T)
Poly-3 test	P = 0.444N	P = 0.295N	P = 0.112N	P = 0.357N
Ovary: Cystadenoma				
Overall rate	3/50 (6%)	1/50 (2%)	5/50 (10%)	4/49 (8%)
Adjusted rate	6.9%	2.5%	10.6%	9.0%
Terminal rate	3/34 (9%)	1/33 (3%)	5/44 (11%)	3/39 (8%)
First incidence (days)	729 (T)	729 (T)	729 (T)	715
Poly-3 test	P = 0.319	P = 0.334N	P = 0.397	P = 0.507
Uterus: Stromal Polyp				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.6%	0.0%	6.4%	2.2%
Terminal rate	2/34 (6%)	0/33 (0%)	3/44 (7%)	1/39 (3%)
First incidence (days)	729 (T)	–	729 (T)	729 (T)
Poly-3 test	P = 0.543N	P = 0.255N	P = 0.533	P = 0.492N
All Organs: Malignant Lymphoma				
Overall rate	7/50 (14%)	9/50 (18%)	6/50 (12%)	4/50 (8%)
Adjusted rate	15.6%	21.9%	12.6%	8.7%
Terminal rate	4/34 (12%)	7/33 (21%)	5/44 (11%)	1/39 (3%)
First incidence (days)	581	506	564	479
Poly-3 test	P = 0.111N	P = 0.317	P = 0.457N	P = 0.249N
All Organs: Benign Neoplasms				
Overall rate	20/50 (40%)	17/50 (34%)	13/50 (26%)	13/50 (26%)
Adjusted rate	45.4%	41.6%	27.3%	29.0%
Terminal rate	17/34 (50%)	16/33 (49%)	12/44 (27%)	12/39 (31%)
First incidence (days)	661	584	564	715
Poly-3 test	P = 0.072N	P = 0.448N	P = 0.055N	P = 0.081N
All Organs: Malignant Neoplasms				
Overall rate	18/50 (36%)	18/50 (36%)	16/50 (32%)	12/50 (24%)
Adjusted rate	39.3%	42.6%	32.8%	25.5%

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Terminal rate	9/34 (27%)	11/33 (33%)	12/44 (27%)	5/39 (13%)
First incidence (days)	581	506	380	479
Poly-3 test	P = 0.057N	P = 0.462	P = 0.330N	P = 0.115N
All Organs: Benign or Malignant Neoplasms				
Overall rate	29/50 (58%)	30/50 (60%)	23/50 (46%)	22/50 (44%)
Adjusted rate	62.9%	70.9%	47.1%	46.8%
Terminal rate	19/34 (56%)	23/33 (70%)	19/44 (43%)	15/39 (39%)
First incidence (days)	581	506	380	479
Poly-3 test	P = 0.026N	P = 0.284	P = 0.090N	P = 0.086N

T = terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and ovary; 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 D-3. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Two-year Gavage Study of Green Tea Extract[a]

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	3	6	1	–
Moribund	10	2	2	6
Natural deaths	3	9	3	5
Survivors				
Died last week of study	–	1	–	–
Terminal kill	34	32	44	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(49)	(49)	(50)
Inflammation	–	1 (2%)	1 (2%)	1 (2%)
Perforation	2 (4%)	4 (8%)	–	–
Muscularis, necrosis	–	1 (2%)	–	–
Gallbladder	(49)	(48)	(50)	(49)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(48)	(40)	(49)	(48)
Serosa, inflammation	–	–	–	1 (2%)
Intestine small, ileum	(49)	(46)	(49)	(48)
Hyperplasia	–	–	1 (2%)	–
Ulcer	–	–	1 (2%)	–
Serosa, inflammation	–	–	–	1 (2%)
Intestine small, jejunum	(48)	(43)	(49)	(48)
Peyer's patch, hyperplasia, lymphoid	2 (4%)	1 (2%)	1 (2%)	–
Serosa, inflammation	–	–	–	1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	–	1 (2%)	–	1 (2%)
Basophilic focus	5 (10%)	1 (2%)	2 (4%)	5 (10%)
Clear cell focus	3 (6%)	1 (2%)	–	–
Eosinophilic focus	23 (46%)	14 (28%)	8 (16%)	3 (6%)

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Fatty change	4 (8%)	1 (2%)	–	–
Hematopoietic cell proliferation	3 (6%)	1 (2%)	7 (14%)	3 (6%)
Hemorrhage	1 (2%)	–	–	–
Infiltration cellular, lymphocyte	5 (10%)	2 (4%)	6 (12%)	4 (8%)
Inflammation	6 (12%)	3 (6%)	6 (12%)	5 (10%)
Mixed cell focus	4 (8%)	1 (2%)	2 (4%)	1 (2%)
Pigmentation	4 (8%)	1 (2%)	–	–
Bile duct, cyst	–	–	1 (2%)	–
Hepatocyte, necrosis	8 (16%)	4 (8%)	3 (6%)	3 (6%)
Kupffer cell, hyperplasia	1 (2%)	–	–	1 (2%)
Serosa, fibrosis	–	–	–	1 (2%)
Serosa, inflammation	–	–	–	1 (2%)
Mesentery	(6)	(0)	(3)	(2)
Artery, inflammation	–	–	1 (33%)	–
Fat, necrosis	5 (83%)	–	1 (33%)	2 (100%)
Pancreas	(50)	(49)	(50)	(50)
Cyst	–	1 (2%)	–	–
Inflammation	–	–	–	1 (2%)
Acinus, atrophy	–	1 (2%)	1 (2%)	1 (2%)
Salivary glands	(50)	(48)	(49)	(48)
Artery, inflammation	–	1 (2%)	–	–
Stomach, forestomach	(49)	(49)	(50)	(49)
Erosion	–	1 (2%)	–	–
Inflammation	–	1 (2%)	–	–
Mineralization	–	1 (2%)	–	–
Epithelium, hyperplasia	–	1 (2%)	1 (2%)	2 (4%)
Stomach, glandular	(49)	(48)	(49)	(49)
Erosion	–	–	–	1 (2%)
Inflammation	–	–	1 (2%)	–
Mineralization	–	–	–	1 (2%)
Ulcer	–	–	–	1 (2%)
Epithelium, pigmentation	–	1 (2%)	–	–
Serosa, inflammation	–	–	–	1 (2%)
Tongue	(0)	(0)	(0)	(2)

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation	–	1 (2%)	–	–
Aorta, inflammation	–	–	2 (4%)	–
Aorta, mineralization	–	–	–	1 (2%)
Carotid artery, inflammation	–	–	1 (2%)	–
Heart	(50)	(49)	(50)	(50)
Cardiomyopathy	12 (24%)	9 (18%)	5 (10%)	8 (16%)
Infiltration cellular, lymphocyte	–	1 (2%)	–	–
Inflammation	1 (2%)	–	–	–
Mineralization	–	1 (2%)	–	1 (2%)
Artery, degeneration	–	–	–	1 (2%)
Artery, inflammation	–	1 (2%)	–	1 (2%)
Valve, thrombosis	–	1 (2%)	–	–
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Hematopoietic cell proliferation	–	–	1 (2%)	–
Hypertrophy	–	1 (2%)	3 (6%)	1 (2%)
Adrenal medulla	(49)	(50)	(50)	(50)
Hyperplasia	–	–	1 (2%)	2 (4%)
Islets, pancreatic	(50)	(49)	(50)	(50)
Hyperplasia	1 (2%)	3 (6%)	–	–
Parathyroid gland	(35)	(40)	(41)	(35)
Pituitary gland	(50)	(48)	(49)	(50)
Pars distalis, hyperplasia	2 (4%)	3 (6%)	1 (2%)	–
Thyroid gland	(50)	(48)	(49)	(49)
Infiltration cellular, lymphocyte	–	–	–	1 (2%)
C-cell, hyperplasia	1 (2%)	–	–	1 (2%)
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(49)	(49)	(50)	(50)
Ovary	(50)	(50)	(50)	(49)
Angiectasis	1 (2%)	–	1 (2%)	1 (2%)
Cyst	3 (6%)	11 (22%)	6 (12%)	4 (8%)
Hyperplasia, tubular	–	–	–	1 (2%)

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Thrombosis	1 (2%)	–	2 (4%)	–
Corpus luteum, hyperplasia	–	1 (2%)	–	–
Uterus	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)	–	1 (2%)	2 (4%)
Inflammation	–	–	–	1 (2%)
Thrombosis	1 (2%)	–	–	–
Endometrium, hyperplasia, cystic	32 (64%)	28 (56%)	19 (38%)	25 (50%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	6 (12%)	11 (22%)	41 (82%)	34 (68%)
Lymph node	(5)	(5)	(3)	(2)
Hyperplasia	–	–	1 (33%)	–
Lumbar, inflammation	–	–	1 (33%)	–
Mediastinal, hyperplasia, lymphoid	1 (20%)	–	–	1 (50%)
Renal, ectasia	2 (40%)	1 (20%)	–	–
Lymph node, mandibular	(50)	(48)	(49)	(48)
Atrophy	3 (6%)	4 (8%)	–	–
Hyperplasia, lymphoid	–	1 (2%)	8 (16%)	12 (25%)
Infiltration cellular, plasma cell	–	–	31 (63%)	18 (38%)
Lymph node, mesenteric	(50)	(49)	(50)	(50)
Atrophy	1 (2%)	1 (2%)	6 (12%)	5 (10%)
Ectasia	1 (2%)	–	–	–
Hyperplasia, lymphoid	2 (4%)	–	–	–
Infiltration cellular, plasma cell	1 (2%)	–	–	1 (2%)
Spleen	(50)	(49)	(50)	(50)
Hematopoietic cell proliferation	35 (70%)	34 (69%)	38 (76%)	30 (60%)
Necrosis	–	1 (2%)	–	–
Pigmentation, hemosiderin	2 (4%)	5 (10%)	3 (6%)	2 (4%)
Lymphoid follicle, hyperplasia	19 (38%)	13 (27%)	14 (28%)	8 (16%)
White pulp, atrophy	8 (16%)	10 (20%)	7 (14%)	6 (12%)
Thymus	(47)	(49)	(49)	(50)
Atrophy	32 (68%)	28 (57%)	27 (55%)	30 (60%)
Hyperplasia, lymphoid	6 (13%)	4 (8%)	8 (16%)	4 (8%)
Necrosis	2 (4%)	4 (8%)	1 (2%)	1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Skin	(50)	(50)	(50)	(50)
Ulcer	1 (2%)	–	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	12 (24%)	15 (30%)	8 (16%)	23 (46%)
Periosteum, fibrosis	–	1 (2%)	–	–
Skeletal muscle	(4)	(0)	(0)	(3)
Nervous System				
Brain	(50)	(49)	(50)	(50)
Necrosis	3 (6%)	–	–	1 (2%)
Arteriole, inflammation	–	–	–	1 (2%)
Respiratory System				
Lung	(50)	(49)	(50)	(50)
Infiltration cellular, lymphocyte	3 (6%)	1 (2%)	3 (6%)	5 (10%)
Inflammation	–	1 (2%)	2 (4%)	–
Thrombosis	–	1 (2%)	–	–
Alveolar epithelium, hyperplasia	6 (12%)	3 (6%)	3 (6%)	2 (4%)
Serosa, inflammation	3 (6%)	5 (10%)	1 (2%)	1 (2%)
Nose	(48)	(48)	(50)	(50)
Foreign body	4 (8%)	8 (17%)	13 (26%)	17 (34%)
Hyperostosis	–	–	21 (42%)	48 (96%)
Infiltration cellular, histiocyte	–	–	–	1 (2%)
Inflammation, suppurative	4 (8%)	24 (50%)	44 (88%)	47 (94%)
Glands, respiratory epithelium, hyperplasia	3 (6%)	–	–	–
Lumen, pigmentation	–	19 (40%)	3 (6%)	16 (32%)
Nasopharyngeal duct, degeneration	–	–	2 (4%)	4 (8%)
Nasopharyngeal duct, inflammation	–	–	1 (2%)	3 (6%)
Nerve, atrophy	–	13 (27%)	47 (94%)	48 (96%)
Olfactory epithelium, accumulation, hyaline droplet	41 (85%)	41 (85%)	3 (6%)	–
Olfactory epithelium, atrophy	–	18 (38%)	26 (52%)	17 (34%)
Olfactory epithelium, fibrosis	–	1 (2%)	39 (78%)	43 (86%)
Olfactory epithelium, metaplasia, respiratory	2 (4%)	36 (75%)	49 (98%)	48 (96%)
Olfactory epithelium, necrosis	–	2 (4%)	1 (2%)	–

	Vehicle Control	30 mg/kg	100 mg/kg	300 mg/kg
Respiratory epithelium, accumulation, hyaline droplet	45 (94%)	42 (88%)	19 (38%)	35 (70%)
Respiratory epithelium, atrophy	–	–	1 (2%)	–
Respiratory epithelium, hyperplasia	1 (2%)	1 (2%)	22 (44%)	15 (30%)
Respiratory epithelium, metaplasia, squamous	–	8 (17%)	42 (84%)	42 (84%)
Respiratory epithelium, necrosis	–	4 (8%)	28 (56%)	32 (64%)
Septum, perforation	–	–	38 (76%)	42 (84%)
Turbinate, atrophy	–	–	40 (80%)	48 (96%)
Trachea	(50)	(48)	(49)	(49)
Special Senses System				
Eye	(50)	(48)	(49)	(50)
Cornea, inflammation	1 (2%)	1 (2%)	1 (2%)	–
Harderian gland	(50)	(46)	(49)	(50)
Hyperplasia	–	3 (7%)	3 (6%)	–
Zymbal's gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	–	2 (4%)	1 (2%)	1 (2%)
Hydronephrosis	–	–	1 (2%)	–
Infarct	3 (6%)	–	1 (2%)	6 (12%)
Infiltration cellular, lymphocyte	1 (2%)	–	1 (2%)	1 (2%)
Mineralization	–	–	–	1 (2%)
Nephropathy	13 (26%)	12 (24%)	11 (22%)	5 (10%)
Artery, inflammation	1 (2%)	–	2 (4%)	1 (2%)
Cortex, cyst	1 (2%)	–	1 (2%)	–
Renal tubule, necrosis	2 (4%)	1 (2%)	–	–
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte	1 (2%)	–	–	–

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

Appendix E. Genetic Toxicology

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Tables

E.1. Bacterial Mutagenicity Test Protocol

Testing procedures were modified from those reported by Zeiger et al.[154]. Samples of green tea extract (the same chemical lot that was used in the 3-month and 2-year studies) were sent to the laboratory as coded aliquots. They were incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 and *Escherichia coli* strain 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 (or tryptophan for the *E. coli* strain) and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine- or tryptophan-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 green tea extract. The high dose was limited by toxicity. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine- or tryptophan-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.

E.2. Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor et al.[155]. At the end of the 3-month 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) in each of four (1,000 mg/kg males) or five mice per dose group. In addition, the percentage of circulating polychromatic erthyrocytes (PCEs; reticulocytes) in 1,000 total erythrocytes per animal was determined to provide a measure of chemical-induced 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 using 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 study were accepted without repeat tests because additional test data could not be observed. 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.

E.3. 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 samples of a chemical were tested in the same assay, and different results were obtained among these samples 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 judgment of the overall evidence for activity of the chemical in an assay.

E.4. Results

Green tea extract (50 to 2,000 µg/plate), from the same lot that was tested in the 3-month and 2-year studies, was mutagenic in *S. typhimurium* strains TA98 and TA100 in the presence of induced rat liver S9; no mutagenicity was observed in these strains without S9 or in the *E. coli* strain WP2 *uvrA/*pKM101 (10 to 1,000 µg/plate) with or without S9 (Table E-1). In vivo, no increases in the frequencies of micronucleated NCEs were seen in peripheral blood of male or female B6C3F1/N mice administered green tea extract (62.5 to 1,000 mg/kg per day) for 3 months by gavage; no significant changes were observed in the percentage of PCEs among total erythrocytes in the blood of either males or females, suggesting that green tea extract did not induce bone marrow toxicity (Table E-2).

Table E-1. Mutagenicity of Green Tea Extract in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9	With 10% Rat S9
TA100						
	0	65 ± 9	39 ± 3	73 ± 4	51 ± 5	69 ± 13
	50	51 ± 2	54 ± 2	72 ± 10	97 ± 9	72 ± 3
	100	62 ± 8	63 ± 7	73 ± 9	105 ± 8	67 ± 5
	500	64 ± 7	55 ± 1	70 ± 7	108 ± 5	124 ± 19
	1,000	45 ± 4	45 ± 4	47 ± 3	149 ± 7	120 ± 7
	2,000	28 ± 6	7 ± 2	121 ± 5	130 ± 11	126 ± 2
Trial summary		Negative	Negative	Negative	Positive	Positive
Positive control[b]		459 ± 5	617 ± 27	740 ± 78	768 ± 36	670 ± 88
TA98						
	0	32 ± 3	24 ± 1	26 ± 3	27 ± 1	
	50	36 ± 2	26 ± 4	31 ± 5	36 ± 2	
	100	31 ± 5	24 ± 1	38 ± 2	40 ± 2	
	500	34 ± 2	45 ± 5	67 ± 5	84 ± 4	
	1,000	30 ± 1	34 ± 1	63 ± 8	115 ± 4	
	2,000	23 ± 6	27 ± 3	66 ± 6	119 ± 8	
Trial summary		Negative	Negative	Positive	Positive	
Positive control		413 ± 14	429 ± 11	840 ± 81	649 ± 49	

***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**

		Without S9	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9	With 10% Rat S9
	0	135 ± 6	153 ± 11	208 ± 6	180 ± 5	149 ± 12	220 ± 14
	10	142 ± 7	148 ± 3	220 ± 6	183 ± 11	166 ± 9	217 ± 3
	50	147 ± 16	147 ± 11	206 ± 5	220 ± 25	178 ± 6	221 ± 11
	100	133 ± 4	158 ± 6	211 ± 8	174 ± 1	177 ± 9	271 ± 27
	500	Toxic	116 ± 12	146 ± 11	130 ± 8	130 ± 6	153 ± 9
	1,000	Toxic	46 ± 4	75 ± 20[c]	Toxic	53 ± 12	95 ± 4
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		804 ± 31	1,101 ± 55	724 ± 12	821 ± 28	734 ± 7	789 ± 19

[a]SITEK Research Laboratories used a modification of the Zeiger et al.[154] protocol and the same lot (GTE50-A0302031114) that was used in the 3-month and 2-year studies. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.
[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.
[c]Slight toxicity.

Table E-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Green Tea Extract by Gavage for Three Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Water[d]	0	5	1.90 ± 0.33		4.40 ± 0.18
Green tea extract	62.5	5	2.60 ± 0.24	0.1481	4.54 ± 0.22
	125	5	2.20 ± 0.34	0.3195	4.50 ± 0.24
	250	5	1.90 ± 0.43	0.5000	4.10 ± 0.11
	500	5	1.70 ± 0.25	0.6307	3.72 ± 0.31
	1,000	4	1.88 ± 0.24	0.5153	4.28 ± 0.30
			P = 0.780[e]		
Female					
Water	0	5	1.50 ± 0.16		3.70 ± 0.14
Green tea extract	62.5	5	1.90 ± 0.33	0.2462	4.00 ± 0.25
	125	5	1.70 ± 0.34	0.3617	3.18 ± 0.17
	250	5	1.40 ± 0.10	0.5737	3.72 ± 0.24
	500	5	1.40 ± 0.19	0.5737	3.44 ± 0.31
	1,000	5	1.20 ± 0.12	0.7183	3.48 ± 0.20
			P = 0.863		

NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.
[a]Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor et al.[155].
[b]Mean ± standard error.
[c]Pairwise comparison with the vehicle control group; dosed group values are significant at $P \leq 0.005$.
[d]Vehicle control.
[e]Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at $P \leq 0.025$.

Appendix F. Clinical Pathology Results

Tables

Table F-1. Hematology and Clinical Chemistry Data for F344/NTac Rats in the Three-month Gavage Study of Green Tea 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	10	10	10
Day 23	10	10	10	10	10	10
Week 14	9	10	10	10	10	10
Hematocrit (%)						
Day 4	44.3 ± 0.4	44.9 ± 0.2	45.6 ± 0.5	46.2 ± 0.5*	44.7 ± 0.4	46.4 ± 0.5*
Day 23	47.1 ± 0.4	46.3 ± 0.4	47.7 ± 0.4	46.5 ± 0.8	45.9 ± 0.6	47.4 ± 0.8
Week 14	45.5 ± 0.6	44.5 ± 0.3	45.4 ± 0.4	45.3 ± 0.2	45.7 ± 0.4	45.6 ± 0.4
Hemoglobin (g/dL)						
Day 4	13.4 ± 0.1	13.6 ± 0.1	13.8 ± 0.2	13.9 ± 0.1	13.4 ± 0.1	13.9 ± 0.2*
Day 23	15.5 ± 0.2	15.0 ± 0.2	15.3 ± 0.1	15.1 ± 0.2	14.8 ± 0.2	15.3 ± 0.3
Week 14	15.3 ± 0.2	15.0 ± 0.1	15.2 ± 0.1	15.1 ± 0.1	15.4 ± 0.1	15.5 ± 0.1
Erythrocytes (10^6/µL)						
Day 4	7.24 ± 0.08	7.42 ± 0.04	7.48 ± 0.08	7.57 ± 0.11	7.33 ± 0.08	7.53 ± 0.11
Day 23	8.25 ± 0.07	8.19 ± 0.07	8.29 ± 0.09	8.22 ± 0.16	8.11 ± 0.10	8.37 ± 0.17
Week 14	9.42 ± 0.14	9.18 ± 0.05	9.34 ± 0.06	9.34 ± 0.05	9.35 ± 0.07	9.46 ± 0.09
Reticulocytes (10^6/µL)						
Day 4	590.6 ± 17.1	511.6 ± 29.7	510.3 ± 31.2	533.4 ± 18.6	534.7 ± 24.9	485.3 ± 16.2**
Day 23	320.5 ± 14.9	303.9 ± 7.7	340.0 ± 11.2	336.9 ± 24.9	331.9 ± 18.1	334.5 ± 13.2
Week 14	227.0 ± 7.1	210.8 ± 8.4	211.0 ± 3.8	224.7 ± 7.0	221.7 ± 4.4	168.7 ± 10.8**
Mean cell volume (fL)						
Day 4	61.3 ± 0.3	60.5 ± 0.3	61.0 ± 0.3	61.0 ± 0.4	61.1 ± 0.3	61.6 ± 0.4
Day 23	57.2 ± 0.2	56.6 ± 0.3	57.5 ± 0.2	56.5 ± 0.3	56.6 ± 0.3	56.7 ± 0.4
Week 14	48.3 ± 0.3	48.5 ± 0.2	48.7 ± 0.3	48.4 ± 0.2	48.9 ± 0.2	48.2 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	18.5 ± 0.1	18.3 ± 0.1	18.4 ± 0.1	18.4 ± 0.1	18.3 ± 0.1	18.5 ± 0.1
Day 23	18.7 ± 0.1	18.3 ± 0.1	18.5 ± 0.1	18.4 ± 0.1	18.3 ± 0.1*	18.3 ± 0.1*
Week 14	16.2 ± 0.1	16.3 ± 0.1	16.3 ± 0.1	16.2 ± 0.1	16.5 ± 0.1	16.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	30.1 ± 0.2	30.2 ± 0.1	30.2 ± 0.2	30.1 ± 0.1	30.0 ± 0.1	30.0 ± 0.2
Day 23	32.8 ± 0.2	32.3 ± 0.1	32.1 ± 0.2	32.5 ± 0.2	32.3 ± 0.2	32.3 ± 0.3
Week 14	33.6 ± 0.2	33.6 ± 0.2	33.5 ± 0.1	33.4 ± 0.2	33.6 ± 0.1	33.9 ± 0.1

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Platelets (10^3/µL)						
Day 4	1,129.3 ± 42.9	1,176.7 ± 37.5	1,181.8 ± 47.9	1,172.9 ± 40.6	1,237.7 ± 36.8	1,232.3 ± 38.9
Day 23	899.7 ± 60.3	946.7 ± 48.5	893.8 ± 62.7	975.9 ± 65.2	1,015.7 ± 52.2	936.4 ± 70.9
Week 14	695.0 ± 63.4	715.1 ± 13.5	736.3 ± 15.8	769.5 ± 22.3	769.4 ± 25.1	799.0 ± 40.0
Leukocytes (10^3/µL)						
Day 4	7.75 ± 0.18	7.09 ± 0.37	7.20 ± 0.29	7.60 ± 0.51	6.57 ± 0.22**	7.11 ± 0.29
Day 23	6.99 ± 0.57	6.60 ± 0.60	6.91 ± 0.46	8.41 ± 0.40	7.92 ± 0.67	6.65 ± 0.49
Week 14	6.95 ± 0.44	5.28 ± 0.47	6.04 ± 0.49	5.73 ± 0.46	6.31 ± 0.40	7.84 ± 0.41
Segmented neutrophils (10^3/µL)						
Day 4	1.27 ± 0.05	1.46 ± 0.08	1.43 ± 0.13	1.83 ± 0.30	1.43 ± 0.05	1.55 ± 0.13
Day 23	1.25 ± 0.11	1.52 ± 0.21	1.46 ± 0.07	2.43 ± 0.32**	2.84 ± 0.52**	1.98 ± 0.29**
Week 14	1.53 ± 0.12	1.11 ± 0.10	1.11 ± 0.09	1.43 ± 0.12	1.64 ± 0.13	2.03 ± 0.15
Lymphocytes (10^3/µL)						
Day 4	6.12 ± 0.18	5.27 ± 0.31*	5.41 ± 0.24*	5.43 ± 0.24*	4.81 ± 0.22**	5.23 ± 0.23**
Day 23	5.43 ± 0.47	4.77 ± 0.47	5.11 ± 0.41	5.59 ± 0.33	4.75 ± 0.44	4.43 ± 0.29
Week 14	5.12 ± 0.34	3.97 ± 0.40	4.69 ± 0.42	4.08 ± 0.36	4.41 ± 0.38	5.53 ± 0.36
Monocytes (10^3/µL)						
Day 4	0.24 ± 0.01	0.23 ± 0.03	0.24 ± 0.02	0.23 ± 0.03	0.22 ± 0.04	0.23 ± 0.03
Day 23	0.13 ± 0.02	0.12 ± 0.02	0.16 ± 0.02	0.20 ± 0.02	0.16 ± 0.02	0.13 ± 0.02
Week 14	0.17 ± 0.02	0.11 ± 0.02	0.13 ± 0.01	0.14 ± 0.02	0.16 ± 0.01	0.20 ± 0.02
Basophils (10^3/µL)						
Day 4	0.064 ± 0.008	0.060 ± 0.009	0.061 ± 0.010	0.047 ± 0.005	0.052 ± 0.006	0.054 ± 0.005
Day 23	0.055 ± 0.004	0.036 ± 0.007	0.073 ± 0.015	0.057 ± 0.008	0.045 ± 0.005	0.031 ± 0.005*
Week 14	0.022 ± 0.004	0.018 ± 0.003	0.023 ± 0.004	0.021 ± 0.003	0.024 ± 0.003	0.025 ± 0.004
Eosinophils (10^3/µL)						
Day 4	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.00
Day 23	0.12 ± 0.02	0.15 ± 0.04	0.11 ± 0.01	0.14 ± 0.03	0.12 ± 0.02	0.08 ± 0.01
Week 14	0.10 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	9.1 ± 0.3	10.1 ± 0.2	9.3 ± 0.4	10.4 ± 0.5	8.2 ± 0.5	9.9 ± 0.8
Day 23	17.5 ± 0.5	12.7 ± 0.6**	13.6 ± 1.1**	13.2 ± 1.0**	10.6 ± 0.6**	11.6 ± 0.5**
Week 14	15.1 ± 0.7	15.2 ± 0.6	15.2 ± 0.6	13.2 ± 0.6	15.2 ± 0.7	15.1 ± 0.9

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Creatinine (mg/dL)						
Day 4	0.48 ± 0.01	0.50 ± 0.00	0.49 ± 0.01	0.51 ± 0.01*	0.51 ± 0.01*	0.52 ± 0.01*
Day 23	0.55 ± 0.02	0.58 ± 0.01	0.58 ± 0.01	0.60 ± 0.01	0.59 ± 0.01	0.60 ± 0.01
Week 14	0.61 ± 0.01	0.62 ± 0.01	0.67 ± 0.02*	0.64 ± 0.02	0.63 ± 0.02	0.63 ± 0.02
Serum glucose (mg/dL)						
Day 4	136 ± 2	129 ± 2	132 ± 4	132 ± 4	125 ± 3*	132 ± 3
Day 23	142 ± 5	135 ± 2	131 ± 3	135 ± 3	134 ± 4	135 ± 3
Week 14	134 ± 4	135 ± 2	135 ± 3	137 ± 5	128 ± 3	134 ± 5
Total protein (g/dL)						
Day 4	5.6 ± 0.0	5.6 ± 0.1	5.8 ± 0.1	5.8 ± 0.1*	5.8 ± 0.1	5.8 ± 0.1
Day 23	6.5 ± 0.1	6.5 ± 0.1	6.7 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.6 ± 0.1
Week 14	7.2 ± 0.1	7.1 ± 0.0	7.1 ± 0.0	6.9 ± 0.0**	7.0 ± 0.1*	6.8 ± 0.1**
Albumin (g/dL)						
Day 4	4.1 ± 0.0	4.1 ± 0.0	4.2 ± 0.1	4.2 ± 0.0	4.2 ± 0.1	4.2 ± 0.1
Day 23	4.6 ± 0.0	4.5 ± 0.0	4.6 ± 0.0	4.4 ± 0.0*	4.5 ± 0.1	4.6 ± 0.1
Week 14	4.6 ± 0.1	4.6 ± 0.0	4.6 ± 0.0	4.5 ± 0.0	4.5 ± 0.1	4.5 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	61 ± 2	64 ± 2	64 ± 3	74 ± 6	71 ± 2**	72 ± 5*
Day 23	54 ± 2	55 ± 2	61 ± 1	52 ± 2	66 ± 2**	63 ± 3**
Week 14	70 ± 4	81 ± 7	73 ± 5	62 ± 1	59 ± 2	75 ± 2
Alkaline phosphatase (IU/L)						
Day 4	652 ± 10	631 ± 16	636 ± 17	637 ± 16	653 ± 18	667 ± 29
Day 23	447 ± 24	523 ± 8*	561 ± 10**	515 ± 15*	556 ± 21**	602 ± 28**
Week 14	243 ± 4	245 ± 6	236 ± 5	230 ± 4	202 ± 5**	215 ± 11**
Creatine kinase (IU/L)						
Day 4	334 ± 25	515 ± 49*	488 ± 93	668 ± 83**	431 ± 38	529 ± 53*
Day 23	248 ± 25	384 ± 53	420 ± 40*	426 ± 59*	355 ± 48	369 ± 44
Week 14	448 ± 106	520 ± 82	380 ± 74	491 ± 83	340 ± 34	508 ± 57
Sorbitol dehydrogenase (IU/L)						
Day 4	13 ± 0	13 ± 1	14 ± 1	15 ± 2	15 ± 1	15 ± 2
Day 23	16 ± 1	16 ± 1	18 ± 1	16 ± 1	15 ± 1	16 ± 0
Week 14	23 ± 2	28 ± 5	26 ± 1	20 ± 1	20 ± 2	19 ± 2

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Bile salts (µmol/L)						
Day 4	23.6 ± 2.2	31.3 ± 2.7	21.7 ± 3.3	21.3 ± 3.3	14.1 ± 2.6	18.8 ± 3.1
Day 23	13.3 ± 2.1	14.6 ± 1.2	9.2 ± 0.7	13.8 ± 2.4	14.6 ± 2.4	13.3 ± 1.3
Week 14	8.7 ± 1.9	7.8 ± 1.2	8.6 ± 1.1	13.6 ± 1.8*	14.3 ± 1.7*	44.3 ± 6.6**
Female						
Hematology						
n						
Day 4	10	10	9	10	10	9
Day 23	10	10	8	10	10	10
Week 14	10	9	9	10	9	10
Hematocrit (%)						
Day 4	45.7 ± 0.7	46.4 ± 0.6	47.3 ± 0.6	46.5 ± 0.8	48.1 ± 0.3**	48.7 ± 0.8**
Day 23	44.5 ± 0.3	44.6 ± 0.4	44.9 ± 0.3	45.6 ± 0.8	45.4 ± 0.6	44.5 ± 0.3
Week 14	44.1 ± 0.3	43.8 ± 0.4	43.6 ± 0.5	43.1 ± 0.3	43.2 ± 0.5	44.7 ± 0.9
Hemoglobin (g/dL)						
Day 4	14.0 ± 0.2	14.2 ± 0.2	14.5 ± 0.2	14.1 ± 0.3	14.7 ± 0.2*	14.9 ± 0.3*
Day 23	15.3 ± 0.1	15.3 ± 0.1	15.4 ± 0.1	15.4 ± 0.2	15.3 ± 0.2	15.1 ± 0.2
Week 14	15.1 ± 0.1	15.1 ± 0.1	14.9 ± 0.1	14.8 ± 0.1	14.7 ± 0.2	15.3 ± 0.3
Erythrocytes (10^6/µL)						
Day 4	7.67 ± 0.13	7.77 ± 0.09	7.90 ± 0.10	7.70 ± 0.13	8.06 ± 0.08*	8.18 ± 0.13*
Day 23	8.11 ± 0.05	8.12 ± 0.06	8.14 ± 0.09	8.26 ± 0.12	8.28 ± 0.10	8.20 ± 0.06
Week 14	8.60 ± 0.05	8.62 ± 0.06	8.56 ± 0.08	8.48 ± 0.06	8.46 ± 0.08	8.83 ± 0.17
Reticulocytes (10^6/µL)						
Day 4	442.9 ± 28.5	429.2 ± 17.8	424.5 ± 21.1	465.5 ± 22.8	447.4 ± 24.1	448.4 ± 25.4
Day 23	200.8 ± 7.5	200.2 ± 6.4	196.2 ± 7.6	213.7 ± 11.1	208.8 ± 15.8	236.2 ± 16.0
Week 14	199.0 ± 9.1	176.3 ± 10.2	181.3 ± 12.3	182.8 ± 5.0	217.8 ± 9.8	189.9 ± 9.6
Mean cell volume (fL)						
Day 4	59.7 ± 0.3	59.7 ± 0.3	59.9 ± 0.2	60.3 ± 0.3	59.7 ± 0.3	59.5 ± 0.4
Day 23	55.0 ± 0.2	54.9 ± 0.2	55.2 ± 0.2	55.2 ± 0.3	54.8 ± 0.3	54.3 ± 0.3
Week 14	51.4 ± 0.3	50.8 ± 0.2	50.9 ± 0.3	50.9 ± 0.2	51.0 ± 0.2	50.6 ± 0.3
Mean cell hemoglobin (pg)						
Day 4	18.3 ± 0.1	18.2 ± 0.1	18.3 ± 0.1	18.2 ± 0.1	18.2 ± 0.1	18.2 ± 0.2
Day 23	18.9 ± 0.1	18.9 ± 0.1	18.9 ± 0.1	18.7 ± 0.1	18.5 ± 0.1*	18.5 ± 0.2
Week 14	17.6 ± 0.1	17.5 ± 0.1	17.4 ± 0.1	17.5 ± 0.1	17.3 ± 0.1	17.3 ± 0.1

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Mean cell hemoglobin concentration (g/dL)						
Day 4	30.6 ± 0.2	30.5 ± 0.2	30.6 ± 0.2	30.2 ± 0.1	30.6 ± 0.2	30.6 ± 0.2
Day 23	34.4 ± 0.1	34.3 ± 0.2	34.3 ± 0.2	33.9 ± 0.2	33.8 ± 0.2	34.0 ± 0.3
Week 14	34.2 ± 0.1	34.4 ± 0.2	34.2 ± 0.2	34.4 ± 0.1	34.0 ± 0.2	34.2 ± 0.2
Platelets (10^3/µL)						
Day 4	1,029.0 ± 40.5	1,031.8 ± 45.0	943.7 ± 46.9	1,019.7 ± 29.8	1,004.4 ± 49.0	1,017.0 ± 47.1
Day 23	851.5 ± 25.0	870.9 ± 51.7	842.1 ± 38.9	874.0 ± 13.5	959.7 ± 34.3	901.8 ± 51.1
Week 14	748.7 ± 30.7	763.0 ± 16.7	799.8 ± 38.2	807.8 ± 29.7	799.4 ± 26.2	812.8 ± 30.3
Leukocytes (10^3/µL)						
Day 4	7.96 ± 0.28	7.84 ± 0.30	6.82 ± 0.53	7.61 ± 0.31	6.82 ± 0.56	6.52 ± 0.60
Day 23	6.60 ± 0.27	5.29 ± 0.47	5.44 ± 0.59	5.70 ± 0.41	6.32 ± 0.44	5.75 ± 0.53
Week 14	5.93 ± 0.25	4.56 ± 0.27	5.49 ± 0.66	5.55 ± 0.59	5.98 ± 0.60	6.55 ± 0.56
Segmented neutrophils (10^3/µL)						
Day 4	1.24 ± 0.08	1.15 ± 0.09	1.11 ± 0.09	1.18 ± 0.07	1.18 ± 0.09	1.30 ± 0.17
Day 23	1.37 ± 0.08	1.26 ± 0.22	1.33 ± 0.22	1.44 ± 0.21	1.51 ± 0.12	2.06 ± 0.32
Week 14	1.40 ± 0.11	1.11 ± 0.12	1.33 ± 0.22	1.26 ± 0.12	1.35 ± 0.20	1.62 ± 0.16
Lymphocytes (10^3/µL)						
Day 4	6.33 ± 0.25	6.30 ± 0.27	5.36 ± 0.42	6.07 ± 0.26	5.27 ± 0.50*	4.91 ± 0.55*
Day 23	4.96 ± 0.21	3.79 ± 0.39	3.89 ± 0.39	4.00 ± 0.44	4.58 ± 0.43	3.44 ± 0.29**
Week 14	4.26 ± 0.17	3.28 ± 0.20	3.93 ± 0.42	4.08 ± 0.48	4.40 ± 0.42	4.63 ± 0.41
Monocytes (10^3/µL)						
Day 4	0.24 ± 0.01	0.25 ± 0.03	0.19 ± 0.03	0.22 ± 0.02	0.21 ± 0.01	0.18 ± 0.02
Day 23	0.14 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.10 ± 0.01	0.12 ± 0.02	0.12 ± 0.02
Week 14	0.16 ± 0.01	0.11 ± 0.01	0.15 ± 0.03	0.13 ± 0.02	0.14 ± 0.03	0.21 ± 0.05
Basophils (10^3/µL)						
Day 4	0.062 ± 0.007	0.061 ± 0.007	0.056 ± 0.010	0.069 ± 0.007	0.055 ± 0.006	0.046 ± 0.006
Day 23	0.029 ± 0.003	0.023 ± 0.003	0.019 ± 0.002	0.024 ± 0.003	0.023 ± 0.004	0.017 ± 0.003*
Week 14	0.022 ± 0.004	0.011 ± 0.002	0.026 ± 0.007	0.025 ± 0.005	0.020 ± 0.005	0.020 ± 0.003
Eosinophils (10^3/µL)						
Day 4	0.08 ± 0.01	0.08 ± 0.02	0.10 ± 0.02	0.08 ± 0.02	0.11 ± 0.03	0.09 ± 0.01
Day 23	0.11 ± 0.01	0.12 ± 0.02	0.09 ± 0.02	0.13 ± 0.03	0.09 ± 0.02	0.13 ± 0.02
Week 14	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 14	10	10	9	10	10	10
Urea nitrogen (mg/dL)						
Day 4	10.1 ± 0.3	10.3 ± 0.4	9.7 ± 0.5	8.7 ± 0.6[b]	8.9 ± 0.3	9.3 ± 0.6[b]
Day 23	13.3 ± 0.5	13.4 ± 0.4	12.2 ± 0.5	11.7 ± 0.6	11.4 ± 0.4	12.5 ± 1.0
Week 14	13.6 ± 0.5	14.9 ± 0.6	14.6 ± 0.3	13.8 ± 0.6	11.1 ± 0.8	14.2 ± 1.2
Creatinine (mg/dL)						
Day 4	0.46 ± 0.02	0.46 ± 0.02	0.47 ± 0.02	0.43 ± 0.02[b]	0.48 ± 0.01	0.50 ± 0.02[b]
Day 23	0.50 ± 0.00	0.55 ± 0.02	0.52 ± 0.02	0.57 ± 0.02*	0.59 ± 0.01**	0.54 ± 0.02
Week 14	0.65 ± 0.03	0.62 ± 0.02	0.61 ± 0.01	0.63 ± 0.02	0.63 ± 0.02	0.63 ± 0.02
Serum glucose (mg/dL)						
Day 4	129 ± 2[c]	133 ± 3[b]	131 ± 3	123 ± 2[c]	123 ± 2[d]	122 ± 3[d]
Day 23	136 ± 2	144 ± 2	138 ± 6	137 ± 4	128 ± 3	136 ± 3
Week 14	129 ± 5	141 ± 6	134 ± 5	128 ± 4	131 ± 5	140 ± 9
Total protein (g/dL)						
Day 4	5.7 ± 0.1[c]	5.7 ± 0.1[b]	5.8 ± 0.1	5.8 ± 0.1[c]	5.8 ± 0.1[d]	5.8 ± 0.1[d]
Day 23	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.0 ± 0.1
Week 14	7.4 ± 0.1	7.1 ± 0.1	7.1 ± 0.1	6.8 ± 0.1**	6.7 ± 0.1**	6.6 ± 0.1**
Albumin (g/dL)						
Day 4	4.2 ± 0.0[c]	4.2 ± 0.0[b]	4.3 ± 0.1*	4.3 ± 0.1[c]	4.3 ± 0.1[d]	4.3 ± 0.0[d]
Day 23	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.1	4.6 ± 0.1	4.5 ± 0.0	4.4 ± 0.0**
Week 14	5.0 ± 0.1	4.8 ± 0.1	4.8 ± 0.0	4.6 ± 0.1**	4.6 ± 0.0**	4.6 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 4	58 ± 2	60 ± 3	62 ± 2	63 ± 2	71 ± 3**	73 ± 4**
Day 23	38 ± 1	41 ± 1	48 ± 2**	45 ± 2**	42 ± 1*	58 ± 3**
Week 14	54 ± 3	58 ± 3	49 ± 3	56 ± 3	55 ± 3	837 ± 772
Alkaline phosphatase (IU/L)						
Day 4	552 ± 11	550 ± 11	540 ± 11	575 ± 11	558 ± 12	520 ± 14
Day 23	380 ± 9	394 ± 6	406 ± 8*	432 ± 13**	457 ± 9**	435 ± 14**
Week 14	209 ± 3	217 ± 8	196 ± 3	202 ± 6	199 ± 3	228 ± 9

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Creatine kinase (IU/L)						
Day 4	434 ± 29[c]	597 ± 62[b]	422 ± 55	449 ± 39[c]	583 ± 95[d]	501 ± 83[d]
Day 23	244 ± 23	274 ± 30	292 ± 34	282 ± 39	244 ± 27	366 ± 48
Week 14	304 ± 41	481 ± 63	379 ± 104	360 ± 77	439 ± 85	615 ± 120
Sorbitol dehydrogenase (IU/L)						
Day 4	13 ± 1	13 ± 0	13 ± 1	14 ± 1	13 ± 1	14 ± 1
Day 23	16 ± 1	16 ± 1	16 ± 1	17 ± 1	16 ± 1	15 ± 0
Week 14	18 ± 1	16 ± 1	15 ± 1	16 ± 1	15 ± 1	16 ± 1
Bile salts (µmol/L)						
Day 4	14.7 ± 2.2	14.9 ± 1.7	13.4 ± 2.2	19.6 ± 2.6	17.6 ± 3.0	17.7 ± 4.3
Day 23	9.3 ± 1.1	9.1 ± 1.1	10.9 ± 1.8	7.8 ± 1.0	7.5 ± 1.1	12.9 ± 1.8
Week 14	9.4 ± 1.3	10.2 ± 1.7	9.7 ± 0.8	14.8 ± 2.7	17.9 ± 3.2*	46.3 ± 26.2**

*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.
[b]n = 9.
[c]n = 8.
[d]n = 7.

Table F-2. Hematology Data for Mice in the Three-month Gavage Study of Green Tea Extract[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
n	10	10	10	10	10	4
Hematocrit (%)	51.9 ± 1.4	51.8 ± 0.5	50.8 ± 0.7	51.4 ± 0.7	50.3 ± 0.7	48.7 ± 0.7
Hemoglobin (g/dL)	16.5 ± 0.5	16.5 ± 0.2	16.2 ± 0.3	16.3 ± 0.3	16.0 ± 0.2	15.5 ± 0.2
Erythrocytes (10^6/µL)	10.76 ± 0.31	10.71 ± 0.12	10.62 ± 0.16	10.68 ± 0.14	10.49 ± 0.16	10.29 ± 0.16
Reticulocytes (10^6/µL)	276.9 ± 11.7	279.6 ± 10.2	297.0 ± 3.7	293.1 ± 9.4	291.8 ± 11.9	291.1 ± 46.1
Mean cell volume (fL)	48.3 ± 0.1	48.4 ± 0.2	47.9 ± 0.2	48.1 ± 0.1	48.0 ± 0.1	47.4 ± 0.2**
Mean cell hemoglobin (pg)	15.3 ± 0.1	15.4 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.3 ± 0.1	15.0 ± 0.0
Mean cell hemoglobin concentration (g/dL)	31.7 ± 0.2	31.8 ± 0.1	31.7 ± 0.2	31.8 ± 0.2	31.9 ± 0.1	31.7 ± 0.1
Platelets (10^3/µL)	986.3 ± 69.6	924.9 ± 44.5	983.2 ± 46.5	1,061.7 ± 64.6	1,019.5 ± 59.8	1,214.3 ± 133.5
Leukocytes (10^3/µL)	4.59 ± 0.34	4.25 ± 0.35	3.81 ± 0.38	3.60 ± 0.31	4.02 ± 0.31	5.02 ± 0.82
Segmented neutrophils (10^3/µL)	0.68 ± 0.07	0.59 ± 0.09	0.57 ± 0.05	0.54 ± 0.05	0.59 ± 0.06	0.63 ± 0.08
Lymphocytes (10^3/µL)	3.68 ± 0.28	3.47 ± 0.26	3.05 ± 0.33	2.93 ± 0.25	3.29 ± 0.26	4.16 ± 0.75
Monocytes (10^3/µL)	0.15 ± 0.02	0.12 ± 0.02	0.11 ± 0.02	0.07 ± 0.01**	0.07 ± 0.01**	0.10 ± 0.02*
Basophils (10^3/µL)	0.012 ± 0.002	0.008 ± 0.002	0.007 ± 0.002	0.004 ± 0.002*	0.006 ± 0.002	0.020 ± 0.006
Eosinophils (10^3/µL)	0.07 ± 0.01	0.08 ± 0.02	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.11 ± 0.03
Female						
n	9	10	10	9	10	6
Hematocrit (%)	51.4 ± 0.8	51.3 ± 0.8	50.4 ± 0.9	53.0 ± 0.8	52.2 ± 0.8	51.3 ± 1.5
Hemoglobin (g/dL)	16.7 ± 0.3	16.5 ± 0.3	16.3 ± 0.3	17.1 ± 0.3	16.8 ± 0.3	16.7 ± 0.6
Erythrocytes (10^6/µL)	10.82 ± 0.16	10.59 ± 0.15	10.54 ± 0.20	11.08 ± 0.15	10.87 ± 0.15	10.77 ± 0.31
Reticulocytes (10^6/µL)	265.1 ± 14.6	311.9 ± 25.4	290.0 ± 18.3	302.2 ± 8.8	259.0 ± 23.6	321.3 ± 12.8
Mean cell volume (fL)	47.5 ± 0.2	48.5 ± 0.1**	47.8 ± 0.2	47.8 ± 0.2	48.1 ± 0.3	47.6 ± 0.3
Mean cell hemoglobin (pg)	15.4 ± 0.1	15.6 ± 0.1	15.5 ± 0.1	15.5 ± 0.1	15.5 ± 0.1	15.5 ± 0.2
Mean cell hemoglobin concentration (g/dL)	32.4 ± 0.1	32.1 ± 0.1	32.4 ± 0.1	32.3 ± 0.2	32.2 ± 0.2	32.5 ± 0.3
Platelets (10^3/µL)	757.7 ± 56.8	732.7 ± 54.6	798.2 ± 61.0	668.0 ± 56.1	785.8 ± 82.1	989.3 ± 114.4
Leukocytes (10^3/µL)	3.80 ± 0.42	3.43 ± 0.28	3.68 ± 0.34	3.91 ± 0.21	3.85 ± 0.21	3.54 ± 0.45
Segmented neutrophils (10^3/µL)	0.51 ± 0.06	0.37 ± 0.04	0.44 ± 0.05	0.46 ± 0.09	0.43 ± 0.04	0.55 ± 0.15
Lymphocytes (10^3/µL)	3.13 ± 0.35	2.95 ± 0.25	3.12 ± 0.30	3.30 ± 0.17	3.27 ± 0.17	2.84 ± 0.41
Monocytes (10^3/µL)	0.07 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.02
Basophils (10^3/µL)	0.010 ± 0.003	0.005 ± 0.002	0.006 ± 0.002	0.011 ± 0.003	0.017 ± 0.004	0.012 ± 0.005
Eosinophils (10^3/µL)	0.08 ± 0.03	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.02	0.08 ± 0.01	0.07 ± 0.02

*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

Tables

Table G-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/NTac Rats in the Three-month Gavage Study of Green Tea Extract[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	340 ± 4	330 ± 4	330 ± 4	318 ± 4**	302 ± 5**	293 ± 4**
Heart						
Absolute	1.04 ± 0.02	1.00 ± 0.02	0.98 ± 0.02	1.00 ± 0.02	0.98 ± 0.03	0.94 ± 0.02**
Relative	3.071 ± 0.030	3.033 ± 0.036	2.966 ± 0.036	3.143 ± 0.070	3.261 ± 0.051*	3.205 ± 0.042*
R. Kidney						
Absolute	1.09 ± 0.01	1.08 ± 0.02	1.09 ± 0.01	1.09 ± 0.02	1.07 ± 0.03	1.04 ± 0.02
Relative	3.216 ± 0.036	3.284 ± 0.042	3.310 ± 0.012	3.433 ± 0.045**	3.557 ± 0.054**	3.559 ± 0.057**
Liver						
Absolute	12.61 ± 0.30	12.13 ± 0.025	12.14 ± 0.23	11.25 ± 0.23**	10.68 ± 0.30**	11.06 ± 0.27**
Relative	37.128 ± 0.659	36.761 ± 0.500	36.865 ± 0.682	35.369 ± 0.489	35.365 ± 0.609	37.751 ± 0.771
Lung						
Absolute	2.30 ± 0.10	1.90 ± 0.07*	2.04 ± 0.08*	2.01 ± 0.09*	1.87 ± 0.10**	1.83 ± 0.06**
Relative	6.775 ± 0.270	5.778 ± 0.227*	6.194 ± 0.266	6.345 ± 0.309	6.172 ± 0.266	6.244 ± 0.233
Spleen						
Absolute	0.722 ± 0.016	0.677 ± 0.011*	0.679 ± 0.014*	0.679 ± 0.012*	0.621 ± 0.007**	0.543 ± 0.011**
Relative	2.126 ± 0.031	2.053 ± 0.025	2.061 ± 0.039	2.135 ± 0.040	2.063 ± 0.035	1.856 ± 0.047**
R. Testis						
Absolute	1.429 ± 0.023	1.422 ± 0.027	1.408 ± 0.028	1.357 ± 0.035	1.367 ± 0.021	1.331 ± 0.013*
Relative	4.210 ± 0.064	4.314 ± 0.094	4.273 ± 0.070	4.271 ± 0.124	4.545 ± 0.098*	4.556 ± 0.058**
Thymus						
Absolute	0.364 ± 0.016	0.354 ± 0.013	0.330 ± 0.014	0.317 ± 0.022*	0.289 ± 0.012**	0.231 ± 0.011**
Relative	1.071 ± 0.040	1.076 ± 0.047	1.003 ± 0.044	0.994 ± 0.067	0.957 ± 0.035	0.790 ± 0.040**
Female						
n	10	10	9	10	10	10
Necropsy body wt	188 ± 2	184 ± 3	184 ± 3	176 ± 2*	179 ± 4*	176 ± 3**
Heart						
Absolute	0.69 ± 0.02	0.68 ± 0.01	0.67 ± 0.01	0.64 ± 0.01*	0.64 ± 0.01*	0.65 ± 0.01*
Relative	3.647 ± 0.072	3.703 ± 0.053	3.634 ± 0.040	3.637 ± 0.065	3.594 ± 0.050	3.722 ± 0.090

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
R. Kidney						
Absolute	0.70 ± 0.01	0.66 ± 0.01	0.70 ± 0.02	0.64 ± 0.01*	0.65 ± 0.02	0.67 ± 0.02
Relative	3.705 ± 0.060	3.603 ± 0.031	3.828 ± 0.084	3.619 ± 0.057	3.647 ± 0.034	3.787 ± 0.070
Liver						
Absolute	6.57 ± 0.014	6.30 ± 0.015	6.37 ± 0.013	5.95 ± 0.015*	6.26 ± 0.016	6.53 ± 0.014
Relative	34.930 ± 0.604	34.257 ± 0.557	34.665 ± 0.618	33.786 ± 0.758	35.041 ± 0.629	37.232 ± 0.554*
Lung						
Absolute	1.34 ± 0.06	1.32 ± 0.06	1.39 ± 0.05	1.21 ± 0.04	1.29 ± 0.04	1.20 ± 0.06
Relative	7.119 ± 0.275	7.190 ± 0.332	7.531 ± 0.202	6.876 ± 0.207	7.236 ± 0.159	6.814 ± 0.262
Spleen						
Absolute	0.495 ± 0.010	0.453 ± 0.011	0.473 ± 0.020	0.457 ± 0.008	0.468 ± 0.009	0.464 ± 0.009
Relative	2.630 ± 0.045	2.463 ± 0.041	2.577 ± 0.117	2.598 ± 0.050	2.627 ± 0.058	2.647 ± 0.060
Thymus						
Absolute	0.277 ± 0.006	0.265 ± 0.015	0.253 ± 0.009	0.251 ± 0.008	0.230 ± 0.009**	0.235 ± 0.015**
Relative	1.474 ± 0.028	1.433 ± 0.064	1.376 ± 0.050	1.428 ± 0.044	1.289 ± 0.050*	1.334 ± 0.075*

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

Table G-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Wistar Han Rats at the Three-month Interim Evaluation in the Two-year Gavage Study of Green Tea Extract[a]

	Vehicle Control	1,000 mg/kg
n	10	10
Male		
Necropsy body wt	391 ± 9	379 ± 13
Heart		
Absolute	1.13 ± 0.04	1.11 ± 0.05
Relative	2.88 ± 0.07	2.93 ± 0.05
R. Kidney		
Absolute	1.28 ± 0.05	1.37 ± 0.05
Relative	3.26 ± 0.08	3.61 ± 0.08**
Liver		
Absolute	14.33 ± 0.52	13.85 ± 0.58
Relative	36.59 ± 0.90	36.54 ± 0.67
Lung		
Absolute	1.93 ± 0.08	2.03 ± 0.10
Relative	4.92 ± 0.18	5.41 ± 0.29
R. Testis		
Absolute	1.893 ± 0.050	1.894 ± 0.054
Relative	4.867 ± 0.186	5.020 ± 0.092
Thymus		
Absolute	0.392 ± 0.024	0.365 ± 0.025
Relative	0.998 ± 0.047	0.966 ± 0.068
Female		
Necropsy body wt	228 ± 3	227 ± 5
Heart		
Absolute	0.75 ± 0.02	0.77 ± 0.02
Relative	3.29 ± 0.09	3.41 ± 0.08
R. Kidney		
Absolute	0.84 ± 0.02	0.88 ± 0.03
Relative	3.67 ± 0.07	3.85 ± 0.07
Liver		
Absolute	8.39 ± 0.24	8.75 ± 0.023
Relative	36.78 ± 0.84	38.64 ± 1.13
Lung		
Absolute	1.45 ± 0.05	1.48 ± 0.06
Relative	6.34 ± 0.18	6.53 ± 0.23
Thymus		
Absolute	0.370 ± 0.015	0.312 ± 0.023*
Relative	1.625 ± 0.064	1.369 ± 0.082*

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

Table G-3. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Gavage Study of Green Tea Extract[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
n	10	10	10	10	10	4
Necropsy body wt	40.7 ± 1.0	38.5 ± 1.1	39.4 ± 1.0	35.4 ± 0.9**	34.1 ± 0.9**	30.8 ± 0.9**
Heart						
Absolute	0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.19 ± 0.01
Relative	5.316 ± 0.217	5.780 ± 0.246	5.435 ± 0.303	5.542 ± 0.242	6.158 ± 0.324	6.291 ± 0.504
R. Kidney						
Absolute	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.29 ± 0.01	0.27 ± 0.01**	0.30 ± 0.01*
Relative	7.535 ± 0.142	8.096 ± 0.129	7.794 ± 0.135	8.187 ± 0.156*	7.943 ± 0.181*	9.573 ± 0.235**
Liver						
Absolute	1.79 ± 0.06	1.74 ± 0.06	1.66 ± 0.07	1.46 ± 0.05**	1.49 ± 0.05**	1.51 ± 0.09**
Relative	43.913 ± 0.537	45.215 ± 0.723	42.085 ± 0.909	41.215 ± 0.536	43.595 ± 0.757	48.868 ± 1.938**
Lung						
Absolute	0.28 ± 0.02	0.32 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	0.27 ± 0.02	0.27 ± 0.02
Relative	6.968 ± 0.551	8.288 ± 0.443	7.381 ± 0.563	8.035 ± 0.616	7.885 ± 0.497	8.879 ± 0.857
Spleen						
Absolute	0.070 ± 0.002	0.071 ± 0.003	0.072 ± 0.004	0.067 ± 0.001	0.067 ± 0.002	0.072 ± 0.003
Relative	1.709 ± 0.027	1.855 ± 0.046	1.826 ± 0.086	1.900 ± 0.047*	1.981 ± 0.055**	2.345 ± 0.086**
R. Testis						
Absolute	0.119 ± 0.002	0.118 ± 0.002	0.119 ± 0.002	0.119 ± 0.002	0.119 ± 0.002	0.117 ± 0.004
Relative	2.929 ± 0.087	3.072 ± 0.085	3.022 ± 0.070	3.366 ± 0.080**	3.499 ± 0.102**	3.789 ± 0.170**
Thymus						
Absolute	0.056 ± 0.005	0.055 ± 0.003	0.057 ± 0.004	0.050 ± 0.003	0.047 ± 0.002	0.037 ± 0.003**
Relative	1.358 ± 0.100	1.418 ± 0.074	1.437 ± 0.071	1.418 ± 0.072	1.375 ± 0.047	1.181 ± 0.053
Female						
n	10	10	10	10	10	6
Necropsy body wt	30.4 ± 0.6	32.9 ± 1.1	26.6 ± 0.7**	26.8 ± 0.7**	24.3 ± 0.4**	26.2 ± 0.3**
Heart						
Absolute	0.17 ± 0.01	0.18 ± 0.01	0.15 ± 0.01	0.16 ± 0.00	0.15 ± 0.01	0.16 ± 0.01
Relative	5.518 ± 0.219	5.606 ± 0.228	5.664 ± 0.203	5.984 ± 0.145	6.306 ± 0.282	5.930 ± 0.364

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
R. Kidney						
Absolute	0.17 ± 0.00	0.18 ± 0.00*	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.01	0.17 ± 0.01
Relative	5.506 ± 0.138	5.613 ± 0.186	6.081 ± 0.159*	6.013 ± 0.174*	6.486 ± 0.158**	6.535 ± 0.276**
Liver						
Absolute	1.23 ± 0.03	1.39 ± 0.04**	1.15 ± 0.04	1.09 ± 0.03*	1.04 ± 0.03**	1.36 ± 0.03
Relative	40.493 ± 0.710	42.256 ± 0.415	43.110 ± 0.447	40.712 ± 0.973	42.658 ± 0.753*	52.036 ± 1.236**
Lung						
Absolute	0.28 ± 0.01	0.30 ± 0.01	0.28 ± 0.02	0.30 ± 0.01	0.30 ± 0.01	0.29 ± 0.01
Relative	9.209 ± 0.538	9.152 ± 0.402	10.686 ± 0.803	11.236 ± 0.529*	12.148 ± 0.382**	11.149 ± 0.546**
Spleen						
Absolute	0.078 ± 0.002	0.094 ± 0.004**	0.081 ± 0.004	0.079 ± 0.003	0.076 ± 0.003	0.089 ± 0.004
Relative	2.589 ± 0.080	2.888 ± 0.144	3.077 ± 0.152*	2.946 ± 0.119*	3.140 ± 0.123**	3.391 ± 0.185**
Thymus						
Absolute	0.061 ± 0.002	0.066 ± 0.004	0.055 ± 0.002	0.055 ± 0.002	0.047 ± 0.002**	0.055 ± 0.004*
Relative	2.005 ± 0.064	2.015 ± 0.103	2.057 ± 0.067	2.032 ± 0.064	1.912 ± 0.077	2.117 ± 0.146

*Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test.
**$P \leq 0.01$.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-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

Tables

Table H-1. Summary of Reproductive Tissue Evaluations for Male F344/NTac Rats in the Three-month Gavage Study of Green Tea Extract[a]

	Vehicle Control	250 mg/kg	500 mg/kg	1,000 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	340 ± 4	318 ± 4**	302 ± 5**	293 ± 4**
L. Cauda epididymis	0.1351 ± 0.0027	0.1387 ± 0.0023	0.1307 ± 0.0036	0.1193 ± 0.0040**
L. Epididymis	0.4100 ± 0.0063	0.4103 ± 0.0051	0.4038 ± 0.0071	0.3702 ± 0.0061**
L. Testis	1.4643 ± 0.0162	1.4329 ± 0.0190	1.4099 ± 0.0161	1.4011 ± 0.0175*
Spermatid measurements				
Spermatid heads (10^6/testis)	211.00 ± 6.70	206.75 ± 5.73	209.75 ± 6.55	198.50 ± 9.08
Spermatid heads (10^6/g testis)	172.1 ± 4.4	169.0 ± 5.0	172.4 ± 4.5	167.3 ± 7.2
Epididymal spermatozoal measurements				
Sperm motility (%)	84.8 ± 0.5	83.7 ± 0.4	83.8 ± 0.4	84.0 ± 0.4
Sperm (10^6/cauda epididymis)	108.90 ± 5.20	117.78 ± 4.57	97.15 ± 5.63	93.40 ± 6.22
Sperm (10^6/g cauda epididymis)	806 ± 33	849 ± 30	746 ± 42	783 ± 48

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's or Williams' test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (spermatid and epididymal spermatozoal measurements).

Table H-2. Estrous Cycle Characterization for Female F344/NTac Rats in the Three-month Gavage Study of Green Tea 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)	188 ± 2	176 ± 2*	179 ± 4	176 ± 3**
Proportion of regular cycling females[b]	9/10	10/10	10/10	6/9
Estrous cycle length (days)	4.7 ± 0.18	4.9 ± 0.06	5.0 ± 0.00	5.4 ± 0.42[c]
Estrous stages (% of cycle)				
Diestrus	58.1	61.3	58.1	65.6
Proestrus	10.6	13.8	18.1	13.1
Estrus	25.0	23.1	22.5	20.6
Metestrus	5.6	1.3	0.6	0.6
Uncertain diagnoses	0.6	0.6	0.6	0.0

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett'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. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated 1,000 mg/kg females spent significantly more time in extended diestrus than did 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 1 of 10 animals.

Table H-3. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Gavage Study of Green Tea Extract[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	40.7 ± 1.0	39.4 ± 1.0	35.4 ± 0.9**	34.1 ± 0.9**
L. Cauda epididymis	0.0155 ± 0.0011	0.0143 ± 0.0013	0.0149 ± 0.0009	0.0121 ± 0.0016
L. Epididymis	0.0436 ± 0.0041	0.0431 ± 0.0017	0.0428 ± 0.0008	0.0412 ± 0.0038
L. Testis	0.1178 ± 0.0061	0.1145 ± 0.0014	0.1117 ± 0.0021	0.1132 ± 0.0013
Spermatid measurements				
Spermatid heads (10^6/testis)	21.99 ± 0.78	20.20 ± 0.79	19.67 ± 0.77	19.55 ± 1.16*
Spermatid heads (10^6/g testis)	221.6 ± 9.7	203.7 ± 8.9	202.8 ± 8.6	218.2 ± 13.7
Epididymal spermatozoal measurements				
Sperm motility (%)	87.1 ± 0.6	87.3 ± 0.4	86.5 ± 0.7	86.0 ± 0.5
Sperm (10^6/cauda epididymis)	20.81 ± 1.42	20.40 ± 1.01	19.75 ± 1.83	21.43 ± 0.97
Sperm (10^6/g cauda epididymis)	1,356 ± 73	1,484 ± 89	1,378 ± 170	2,164 ± 386

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's test.
**Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test.
[a]Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid heads/g testis and epididymal spermatozoal measurements).

Table H-4. Estrous Cycle Characterization for Female Mice in the Three-month Gavage Study of Green Tea Extract[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	30.4 ± 0.6	26.6 ± 0.7**	26.8 ± 0.7**	24.3 ± 0.4**
Proportion of regular cycling females[b]	9/10	10/10	9/10	9/10
Estrous cycle length (days)	4.5 ± 0.13	3.9 ± 0.05**	4.2 ± 0.10	4.3 ± 0.14
Estrous stages (% of cycle)				
Diestrus	35.6	28.1	30.6	37.5
Proestrus	1.3	0.0	0.0	0.0
Estrus	41.3	46.9	47.5	41.3
Metestrus	21.9	25.0	21.9	21.3

**Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test (body weights) or Dunn's test (estrous cycle length).
[a]Necropsy body weights and estrous cycle length data are presented as mean ± standard error. 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. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated 500 mg/kg females spent significantly more time in extended diestrus than did the vehicle controls.
[b]Number of females with a regular cycle/number of females cycling.

Appendix I. Chemical Characterization and Dose Formulation Studies

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I.1. Procurement and Characterization of Green Tea Extract

Green tea extract was obtained from Amax NutraSource, Inc. (Eugene, OR), in one lot (GTE50-A0302031114) that was used in the 3-month and 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle Chemistry Support Services, a division of Battelle Columbus Operations (Columbus, OH) and by the study laboratory at Battelle Columbus Operations. Reports on analyses performed in support of the green tea extract studies are on file at the National Institute of Environmental Health Sciences.

Lot GTE50-A0302031114 of the chemical, a light-brown powder, was identified as green tea extract by the analytical chemistry laboratory and the study laboratory using infrared spectroscopy. All spectra were consistent with spectra obtained from another lot, and the overall absorbances and spectral composition were consistent with the components that make up typical green tea extract. A representative infrared spectrum is presented in Figure I-1.

The moisture content of lot GTE50-A0302031114 was determined by the analytical chemistry and study laboratories using weight loss on drying; moisture content was also determined by Prevalere Life Sciences, Inc. (Whitesboro, NY), and Galbraith Laboratories (Knoxville, TN) using Karl Fischer titration. The analytical chemistry laboratory determined the purity of the test article using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. Components of the purity profile were identified by comparing the retention times from a chromatogram of lot GTE50-A0302031114 to a chromatogram of a solution containing nine potential green tea extract components; these components were quantified by standard addition using authentic standards (Aldrich, Milwaukee, WI, or Sigma, St. Louis, MO). HPLC with mass spectrometry (MS) detection was used to confirm the identity of these components.

For lot GTE50-A0302031114, weight loss on drying conducted at two laboratories indicated 4.77% and 5.8% water. Karl Fischer titration conducted at one laboratory indicated 6% to 11.6% water and similar analyses conducted at a second laboratory indicated 3% to 4% water; the latter values were consistent with the 3.32% water indicated in the manufacturer's Certificate of Analysis. HPLC/UV using system A indicated eight components with areas greater than or equal to 1% of the total peak area (Table I-1). Six of these peaks were tentatively identified by matching retention times as epicatechin, catechin gallate, caffeine, epigallocatechin gallate, gallocatechin gallate, and epicatechin gallate. The total area of the major polyphenol peaks represented approximately 84.8% of the total peak area; caffeine constituted 12.27% of the total area, and the remainder was two unidentified components. Nine components of the test article HPLC purity profile were identified and quantified by standard addition using the same chromatography system; these components were gallocatechin, epigallocatechin, catechin, epicatechin, catechin gallate, caffeine, epigallocatechin gallate, gallocatechin gallate, and epicatechin gallate. HPLC/MS using system B confirmed the identity of eight of the nine components; epigallocatechin was not identified due to the concentration being below the detection limit in the test article sample. The weight percentage contents of epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin, and caffeine were determined to be 48.4%, 12.8%, 2.26%, 2.83%, and 4.99%, respectively; these values were comparable to those listed in the manufacturer's Certificate of Analysis (53.11%, 13.7%, 2.88%, 3.97%, and 5.42%, respectively). Other catechin polyphenols that were quantified included gallocatechin, catechin, catechin gallate, gallocatechin gallate, and the weight percentage contents were determined to be 0.52%, 0.51%, 0.45%, and 4.6%, respectively. Taken together, these data indicate that lot

GTE50-A0302031114 of the test material was green tea extract. One aliquot of green tea extract was submitted to Covance Laboratories, Inc., (Madison, WI) for nutritional and contaminant testing using standard methods. For this lot, heavy metals present in the extract included antimony (<10 ppb), arsenic (43.9 ppb), cadmium (<10 ppb), lead (9.79 ppb), and mercury (<10 ppb). For mycotoxin analysis, levels of ochratoxin and zearalinine were less than 5 and 50 ppb, respectively; levels for aflatoxins B1, B2, G1, and G2 could not be quantified due to matrix interferences. The pesticide screen also experienced significant matrix effects that prevented quantitation of analytes.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using HPLC/UV by system C. These studies indicated that green tea extract was stable as a bulk chemical for at least 14 days when stored in sealed amber glass containers at temperatures up to 60°C. To ensure stability, the bulk chemical was stored at room temperature in sealed amber glass containers. Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 3-month and 2-year studies using HPLC/UV by system A, and no degradation of the bulk chemical was detected.

I.2. Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing green tea extract with deionized water to give the required concentrations (Table I-2). The dose formulations were stored at room temperature in sealed clear glass bottles, enclosed in amber plastic bags for up to 8 (3-month studies) or 22 (2-year studies) days.

The analytical chemistry laboratory performed a gavageability study of the 200 mg/mL dose formulation using a 25-gauge needle and stability studies of the 6.25 mg/mL dose formulation using HPLC/UV by system C (Table I-1). Gavageability was confirmed and stability was confirmed for at least 42 days for dose formulations stored in sealed amber glass bottles, at room temperature and for at least 3 hours under simulated animal room conditions.

The study laboratory performed gavageability studies of the 100 and 200 mg/mL dose formulations using 20- and 18-gauge ball-tipped stainless steel needles, respectively; in addition, homogeneity studies of the 6.25, 12.5, 100, and 200 mg/mL dose formulations and stability studies of the 6.25, 25, and 200 mg/mL dose formulations were performed using HPLC/UV by system A. Gavageability and homogeneity were confirmed. Stability was confirmed for at least 22 days for dose formulations prepared with sterile water, bottles, caps, and stir bars; the formulas were stored in clear glass bottles sealed in amber plastic bags at room temperature.

Periodic analyses of the dose formulations of green tea extract were conducted by the study laboratory using HPLC/UV by system A. During the 3-month studies, the dose formulations were analyzed three times for EGCG concentraations; all 18 dose formulations were within 10% of the target concentrations (Table I-3). Animal room samples of these dose formulations were also analyzed; all 15 for rats and 14 of 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 2 to 3 months; animal room samples were also analyzed (Table I-4). Of the dose formulations analyzed, all 63 for rats and all 33 for mice were within 10% of the target concentrations; all 12 animal room samples for rats and 12 of 13 for mice were within 10% of the target concentrations.

Table I-1. High-Performance Liquid Chromatography Systems Used in the Gavage Studies of Green Tea Extract[a]

Detection System	Column	Solvent System
System A		
Ultraviolet (274 nm) light	Aqua® C_{18}, 150 mm × 4.6 mm, 3 µm (Phenomenex, Torrance, CA)	A) Milli-Q water with 0.1% formic acid and B) acetonitrile with 0.1% formic acid; linear gradient from 97% A:3% B to 90% A:10% B in 30 minutes, then linear gradient to 70% A:30% B in 10 minutes, held for 10 minutes, then linear gradient to 97% A:3% B in 1 minute, held for 9 minutes; flow rate 1.0 mL/minute
System B		
Mass spectrometry	Aqua® C_{18}, 150 mm × 4.6 mm, 3 µm (Phenomenex)	A) Milli-Q water with 0.1% formic acid and B) acetonitrile with 0.1% formic acid; linear gradient from 97% A:3% B to 90% A:10% B in 30 minutes, then linear gradient to 70% A:30% B in 10 minutes, held for 10 minutes, then linear gradient to 97% A:3% B in 1 minute, held for 14 minutes; flow rate 1.0 mL/minute
System C		
Ultraviolet (274 nm) light	Aqua® C_{18}, 150 mm × 4.6 mm, 3 µm (Phenomenex)	A) 97:3:0.1 Milli-Q water:acetonitrile:formic acid and B) 70:30:0.1 Milli-Q water:acetonitrile:formic acid; linear gradient from 100% A to 75% A:25% B in 30 minutes, then linear gradient to 100% B in 10 minutes, held for 10 minutes, then linear gradient to 100% A in 1 minute, held for 14 minutes; flow rate 1.0 mL/minute

[a]The high-performance liquid chromatographs were manufactured by Waters Corporation (Milford, MA) or Agilent Technologies (Palo Alto, CA). The mass spectrometer was manufactured by Micromass UK Ltd. (Manchester, England).

Table I-2. Preparation and Storage of Dose Formulations in the Gavage Studies of Green Tea Extract

Three-month Studies	Two-year Studies
Preparation	
To prepare the 6.25 mg/mL dose formulation, the specified amount of green tea extract was weighed into a beaker and then analytically transferred to a calibrated glass bottle with at least three rinses of deionized water. For the 12.5 through 200 mg/mL dose formulations, the appropriate amount of test article was weighed directly into a calibrated glass bottle. For all formulations, deionized water was added to approximately half the final bottle volume, the bottle was capped and shaken for approximately 2 minutes, inverted at least 10 times, diluted to final volume with deionized water, capped, and sonicated (kept below 35°C) for at least 15 minutes or until the test article was dissolved, then shaken vigorously by hand for approximately 1 minute and stirred with an overhead stirrer for approximately 5 minutes. The dose formulations were prepared approximately weekly.	To prepare the 3 mg/mL dose formulation, the specified amount of green tea extract was weighed into a beaker and then analytically transferred to a calibrated glass bottle with at least three rinses of deionized water. For the 10 through 200 mg/mL dose formulations, the appropriate amount of test article was weighed directly into a calibrated glass bottle. For all formulations, deionized water was added to approximately half the final bottle volume and the bottles were further processed as described for the 3-month studies. Due to extensive foaming during the final dilution of the 200 mg/mL formulation, the volume of this formulation was confirmed by weighing the formulation and using the density to verify the correct volume. Sterile materials (water, beakers, bottles, caps, and stir bars) were used throughout these procedures. The dose formulations were prepared approximately every 3 weeks.
Chemical Lot Number	
GTE50-A0302031114	GTE50-A0302031114
Maximum Storage Time	
8 days	22 days
Storage Conditions	
Stored in sealed clear glass bottles enclosed in amber plastic bags at room temperature	Stored in clear glass bottles sealed in amber plastic bags at room temperature
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

Table I-3. Results of Analyses of Dose Formulations Administered to F344/NTac Rats and B6C3F1/N Mice in the Three-month Gavage Studies of Green Tea Extract

Date Prepared	Date Analyzed[a]	Target Concentration (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
Rats				
April 14, 2006	April 13–16, 2006	12.5	12.50	0
		25	24.89	0
		50	50.60	+1
		100	103.5	+4
		200	198.6	−1
	April 27–29, May 1, 2006[c]	12.5	12.12	−3
		25	24.93	0
		50	49.53	−1
		100	98.33	−2
		200	194.8	−3
June 2, 2006	June 1–3, 2006	12.5	12.39	−1
		25	24.78	−1
		50	49.48	−1
		100	98.72	−1
		200	192.3	−4
	June 12–14, 2006[c]	12.5	12.49	0
		25	25.05	0
		50	49.27	−1
		100	101.1	+1
		200	198.9	−1
July 12, 2006	July 12–14, 2006	12.5	12.19	−2
		25	24.06	−4
		50	47.85	−4
		100	96.30	−4
		200	208.9	+4
	July 31–August 2, 2006[c]	12.5	12.50	0
		25	24.29	−3
		50	49.66	−1
		100	99.40	−1
		200	210.6	+5

Date Prepared	Date Analyzed[a]	Target Concentration (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
Mice				
April 14, 2006	April 13–16, 2006	6.25	6.335	+1
		12.5	12.50	0
		25	24.89	0
		50	50.60	+1
		100	103.5	+4
	April 27−29, May 1, 2006[c]	6.25	5.987	−4
		12.5	12.07	−3
		25	24.65	−1
		50	49.75	−1
		100	99.15	−1
June 2, 2006	June 1–3, 2006	6.25	6.165	−1
		12.5	12.39	−1
		25	24.78	−1
		50	49.48	−1
		100	98.72	−1
	June 12–14, 2006[c]	6.25	6.128	−2
		12.5	12.45	0
		25	25.04	0
		50	49.11	−2
		100	100.3	0
July 12, 2006	July 12–14, 2006	6.25	6.123	−2
		12.5	12.19	−2
		25	24.06	−4
		50	47.85	−4
		100	96.30	−4
	July 31–August 2, 2006[c]	6.25	1.027[d]	−84
		12.5	13.15	+5
		25	25.95	+4
		50	52.50	+5
		100	106.7	+7

[a]Due to the length of the analytical runs, the date of analysis may precede the date of preparation.
[b]Results of duplicate analyses. For rats, dosing volume = 5 mL/kg; 12.5 mg/mL = 62.5 mg/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. For mice, dosing volume = 10 mL/kg; 6.25 mg/mL = 62.5 mg/kg, 12.5 mg/mL = 125 mg/kg, 25 mg/mL = 250 mg/kg, 50 mg/mL = 500 mg/kg, 100 mg/mL = 1,000 mg/kg.
[c]Animal room samples.
[d]Results of four replicate analyses; evidence indicated that the sample was hydrolyzed.

Table I-4. Results of Analyses of Dose Formulations Administered to Wistar Han Rats and B6C3F1/N Mice in the Two-year Gavage Studies of Green Tea Extract

Date Prepared	Date Analyzed[a]	Target Concentration (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
Rats				
July 5, 2007	July 3–5, 2007	20	21.1	+6
		20	21.1	+6
		60	62.9	+5
		60	60.9	+2
		200	193	−4
		200	204	+2
	August 2, 2007[c]	20	20.3	+2
		60	62.5	+4
		200	191	−5
September 6, 2007	September 6, 2007	20	20.5	+3
		20	20.5	+3
		60	60.6	+1
		60	61.3	+2
		200	216	+8
September 11, 2007	September 10, 2007	200	214	+7
November 8, 2007	November 7–8, 2007	20	20.5	+3
		20	20.6	+3
		60	61.8	+3
		60	60.8	+1
		200	206	+3
		200	206	+3
January 31, 2008	January 30–31, 2008	20	21.2	+6
		20	20.8	+4
		60	62.1	+4
		60	61.5	+3
		200	208	+4
		200	209	+5
	February 22, 2008[c]	20	20.9	+5
		60	61.7	+3
		200	207	+4
April 3, 2008	April 2, 2008	20	20.3	+2
		20	20.6	+3

Date Prepared	Date Analyzed[a]	Target Concentration (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
		60	63.0	+5
		60	64.0	+7
		200	193	−4
		200	200	0
June 5, 2008	June 4, 2008	20	21.0	+5
		20	20.4	+2
		60	61.5	+3
		60	59.7	−1
		200	209	+5
		200	208	+4
August 28, 2008	August 27, 2008	20	20.9	+5
		20	20.0	0
		60	63.4	+6
		60	61.8	+3
		200	206	+3
		200	198	−1
	September 19, 2008[c]	20	20.1	+1
		60	62.0	+3
		200	206	+3
October 30, 2008	October 29, 2008	20	20.9	+5
		20	21.3	+7
		60	63.1	+5
		60	64.4	+7
		200	214	+7
		200	215	+8
January 22, 2009	January 21, 2009	20	20.6	+3
		20	20.8	+4
		60	62.7	+5
		60	60.9	+2
		200	209	+5
March 26, 2009	March 26, 2009	20	21.0	+5
		20	21.1	+6
		60	62.6	+4
		60	63.4	+6
		200	208	+4

Date Prepared	Date Analyzed[a]	Target Concentration (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
	April 20, 2009[c]	20	20.2	+1
		60	61.9	+3
		200	210	+5
June 18, 2009	June 17, 2009	20	20.7	+4
		20	21.8	+9
		60	63.4	+6
		60	64.5	+8
		200	211	+6
Mice				
July 5, 2007	July 3, 2007	3	3.10	+3
		10	10.4	+4
		30	31.3	+4
	August 2, 2007[c]	3	1.61[d]	−46
	August 16, 2007[c]	10	10.3	+3
		30	29.9	0
July 26, 2007	August 16, 2007[c]	3	2.76	−8
September 6, 2007	September 6, 2007	3	3.05	+2
		10	10.2	+2
		30	30.8	+3
November 8, 2007	November 7–8, 2007	3	3.07	+2
		10	10.3	+3
		30	29.8	−1
January 31, 2008	January 30, 2008	3	3.08	+3
		10	10.5	+5
		30	31.4	+5
	February 22, 2008[c]	3	2.89	−4
		10	10.3	+3
		30	30.7	+2
April 3, 2008	April 2, 2008	3	3.02	+1
		10	10.0	0
		30	30.1	0
June 5, 2008	June 4, 2008	3	3.14	+5
		10	10.4	+4
		30	31.0	+3
August 28, 2008	August 27, 2008	3	3.07	+2

Date Prepared	Date Analyzed[a]	Target Concentration (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
		10	9.98	0
		30	30.1	0
	September 19, 2008[c]	3	2.96	−1
		10	10.0	0
		30	30.9	+3
October 30, 2008	October 29, 2009	3	3.05	+2
		10	10.4	+4
		30	30.9	+3
January 22, 2009	January 21, 2009	3	3.05	+2
		10	10.2	+2
		30	31.0	+3
March 26, 2009	March 26, 2009	3	2.94	−2
		10	10.1	+1
		30	31.0	+3
	April 20, 2009[c]	3	3.02	+1
		10	9.83	−2
		30	30.1	0
June 18, 2009	June 17, 2009	3	3.09	+3
		10	10.5	+5
		30	31.0	+3

[a]Due to the length of the analytical runs, the date of analysis may precede the date of preparation.
[b]Results of duplicate analyses. For rats, dosing volume = 5 mL/kg; 20 mg/mL = 100 mg/kg, 60 mg/mL = 300 mg/kg, 200 mg/mL = 1,000 mg/kg. For mice, dosing volume = 10 mL/kg; 3 mg/mL = 30 mg/kg, 10 mg/mL = 100 mg/kg, 30 mg/mL = 300 mg/kg.
[c]Animal room samples.
[d]Sample contained a fungal growth.

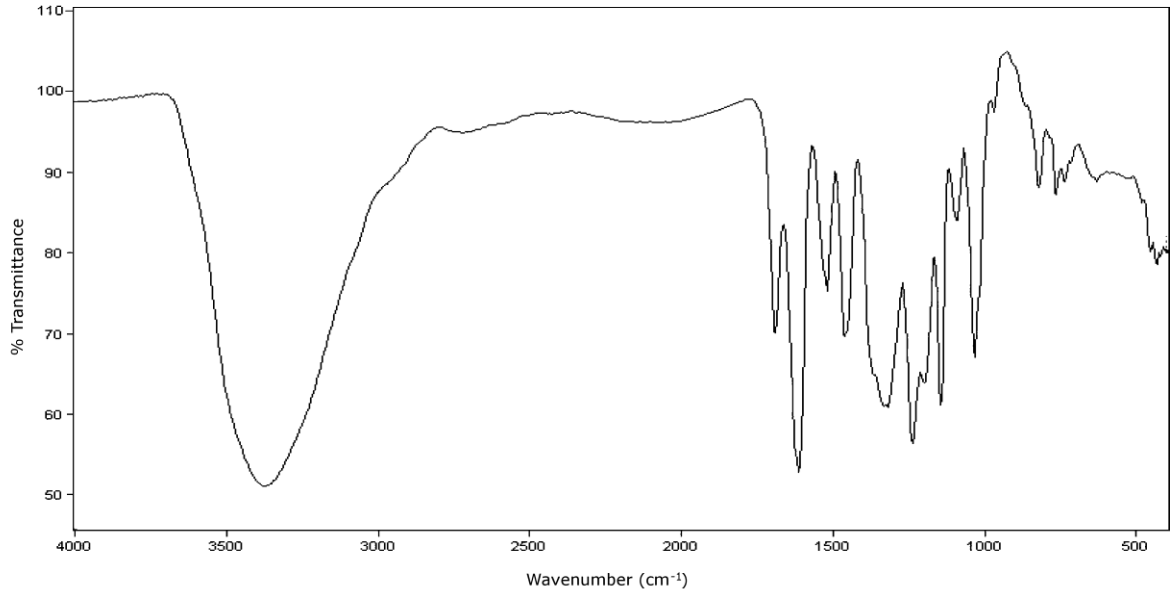

Figure I-1. Infrared Absorption Spectrum of Green Tea Extract

Appendix J. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

Table J-1. 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 J-2. 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 J-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.7 ± 0.65	13.7–15.9	23
Crude fat (% by weight)	8.2 ± 0.28	7.7–8.8	23
Crude fiber (% by weight)	9.1 ± 0.53	8.2–10.3	23
Ash (% by weight)	5.1 ± 0.21	4.4–5.4	23
Amino Acids (% of total diet)			
Arginine	0.786 ± 0.070	0.670–0.970	23
Cystine	0.220 ± 0.024	0.150–0.250	23
Glycine	0.700 ± 0.040	0.620–0.800	23
Histidine	0.351 ± 0.076	0.270–0.680	23
Isoleucine	0.546 ± 0.043	0.430–0.660	23
Leucine	1.095 ± 0.066	0.960–1.240	23
Lysine	0.705 ± 0.116	0.310–0.860	23
Methionine	0.409 ± 0.045	0.260–0.490	23
Phenylalanine	0.628 ± 0.039	0.540–0.720	23
Threonine	0.506 ± 0.042	0.430–0.610	23
Tryptophan	0.150 ± 0.028	0.110–0.200	23
Tyrosine	0.405 ± 0.063	0.280–0.540	23
Valine	0.664 ± 0.043	0.550–0.730	23
Essential Fatty Acids (% of total diet)			
Linoleic	3.95 ± 0.254	3.49–4.55	23
Linolenic	0.30 ± 0.031	0.21–0.35	23
Vitamins			
Vitamin A (IU/kg)	3,641 ± 81	2,350–5,720	23
Vitamin D (IU/kg)	1,000[a]	–	
α-Tocopherol (ppm)	80.3 ± 21.56	27.0–124.0	23
Thiamine (ppm)[b]	6.9 ± 1.10	5.1–9.0	23
Riboflavin (ppm)	7.7 ± 2.87	4.20–17.50	23
Niacin (ppm)	79.2 ± 8.97	66.4–98.2	23
Pantothenic acid (ppm)	27.0 ± 12.35	17.4–81.0	23
Pyridoxine (ppm)[b]	9.54 ± 1.94	6.44–13.7	23
Folic acid (ppm)	1.61 ± 0.47	1.15–3.27	23
Biotin (ppm)	0.32 ± 0.10	0.20–0.704	23
Vitamin B_{12} (ppb)	53.4 ± 38.0	18.3–174.0	23
Choline (ppm)[b]	2,773 ± 590	1,160–3,790	23

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.920 ± 0.048	0.808–1.020	23
Phosphorus (%)	0.556 ± 0.067	0.471–0.822	23
Potassium (%)	0.667 ± 0.030	0.626–0.733	23
Chloride (%)	0.385 ± 0.038	0.300–0.474	23
Sodium (%)	0.189 ± 0.016	0.160–0.222	23
Magnesium (%)	0.216 ± 0.061	0.185–0.490	23
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	187 ± 38.6	135–311	23
Manganese (ppm)	51.0 ± 10.19	21.0–73.1	23
Zinc (ppm)	53.6 ± 8.34	43.3–78.5	23
Copper (ppm)	7.10 ± 2.540	3.21–16.3	23
Iodine (ppm)	0.503 ± 0.201	0.158–0.972	23
Chromium (ppm)	0.696 ± 0.269	0.330–1.380	23
Cobalt (ppm)	0.248 ± 0.163	0.094–0.864	21

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

Table J-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.23 ± 0.040	0.16–0.32	23
Cadmium (ppm)	0.06 ± 0.010	0.05–0.10	23
Lead (ppm)	0.10 ± 0.021	0.07–0.16	23
Mercury (ppm)	<0.02	–	23
Selenium (ppm)	0.23 ± 0.175	0.14–1.02	23
Aflatoxins (ppb)	<5.00	–	23
Nitrate nitrogen (ppm)[c]	20.2 ± 8.48	10.0–42.3	23
Nitrite nitrogen (ppm)[c]	<0.61	–	23
BHA (ppm)[d]	<1.0	–	23
BHT (ppm)[d]	<1.0	–	23
Aerobic plate count (CFU/g)	10 ± 0.0	10	23
Coliform (MPN/g)	3.0 ± 0.0	3.0	23
Escherichia coli (MPN/g)	<10	–	23
Salmonella (MPN/g)	Negative	–	23
Total nitrosoamines (ppb)[e]	10.5 ± 6.24	2.0–28.0	23
N-Nitrosodimethylamine (ppb)[e]	3.1 ± 3.35	0.9–11.1	23
N-Nitrosopyrrolidine (ppb)[e]	7.9 ± 4.65	1.0–17.7	23
Pesticides (ppm)			
α-BHC	<0.01	–	23
β-BHC	<0.02	–	23
γ-BHC	<0.01	–	23
δ-BHC	<0.01	–	23
Heptachlor	<0.01	–	23
Aldrin	<0.01	–	23
Heptachlor epoxide	<0.01	–	23
DDE	<0.01	–	23
DDD	<0.01	–	23
DDT	<0.01	–	23
HCB	<0.01	–	23
Mirex	<0.01	–	23
Methoxychlor	<0.05	–	23
Dieldrin	<0.01	–	23
Endrin	<0.01	–	23

	Mean ± Standard Deviation[b]	Range	Number of Samples
Telodrin	<0.01	–	23
Chlordane	<0.05	–	23
Toxaphene	<0.10	–	23
Estimated PCBs	<0.20	–	23
Ronnel	<0.01	–	23
Ethion	<0.02	–	23
Trithion	<0.05	–	23
Diazinon	<0.10	–	23
Methyl chlorpyrifos	0.080 ± 0.074	0.020–0.300	23
Methyl parathion	<0.02	–	23
Ethyl parathion	<0.02	–	23
Malathion	0.066 ± 0.057	0.020–0.234	23
Endosulfan I	<0.01	–	23
Endosulfan II	<0.01	–	23
Endosulfan sulfate	<0.03	–	23

[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

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Tables

K.1. Methods

Rodents used in 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 test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected and allowed to clot and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter spp*. All samples were processed appropriately and evaluated for the presence of pathogens. Samples were sent to BioReliance Corporation (Rockville, MD) or the Research Animal Diagnostic Laboratory (RADIL; University of Missouri, Columbia, MO). 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.

Table K-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program

Method and Test	Time of Collection
Rats	
Three-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
RCV/SDA	1 month
Two-year Study	
ELISA	
PVM	Study start and 1 month
RCV/SDA	Study start and 1 month
Sendai	Study start and 1 month
Immunofluorescence Assay	
Parvovirus	Study start and 1 month
Multiplex Fluorescent Immunoassay	
H-1 (Toolan's H-1 virus)	6, 12, and 18 months, study termination
KRV (Kilham rat virus)	6, 12, and 18 months, study termination
Mycoplasma pulmonis	6, 12, and 18 months, study termination

Method and Test	Time of Collection
Parvovirus NS-1	6, 12, and 18 months, study termination
PVM	6, 12, and 18 months, study termination
RCV/SDA	6, 12, and 18 months, study termination
RMV (rat minute virus)	6, 12, and 18 months, study termination
RPV (rat parvovirus)	6, 12, and 18 months, study termination
RTV (rat theliovirus)	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
TMEV (Theiler's murine encephalomyelitis virus)	6, 12, and 18 months, study termination
Mice	
Three-month Study	
ELISA	
Ectromelia virus	Study start, 1 month, study termination
EDIM (epizootic diarrhea of infant mice)	Study start, 1 month, study termination
GDVII (mouse encephalomyelitis virus)	Study start, 1 month, study termination
LCM (lymphocytic choriomeningitis virus)	Study start, 1 month, study termination
Mouse adenoma virus-FL	Study start, 1 month, study termination
MHV (mouse hepatitis virus)	Study start, 1 month, study termination
MMV VP2 (mouse minute virus viral protein 2)	Study start, 1 month, study termination
MPV VP2 (mouse parvovirus viral protein 2)	Study start, 1 month, study termination
PVM	Study start, 1 month, study termination
Reovirus 3	Study start, 1 month, study termination
Sendai	Study start, 1 month, study termination
Two-year Study	
ELISA	
Ectromelia virus	Study start and 1 month
EDIM	Study start and 1 month
GDVII	Study start and 1 month
LCM	Study start and 1 month
Mouse adenoma virus-1	Study start and 1 month
MHV	Study start and 1 month
MMV VP2	Study start and 1 month
MPV VP2	Study start and 1 month
PVM	Study start and 1 month
Reovirus 3	Study start and 1 month
Sendai	Study start and 1 month

Method and Test	Time of Collection
Multiplex Fluorescent Immunoassay	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
MHV	6, 12, and 18 months, study termination
MMV	6, 12, and 18 months, study termination
MNV (mouse norovirus)	6, 12, and 18 months, study termination
MPV	6, 12, and 18 months, study termination
M. pulmonis	6, 12, and 18 months, study termination
Parvovirus NS-1	6, 12, and 18 months, study termination
PVM	6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
TMEV GDVII	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Polymerase Chain Reaction	
Helicobacter spp.	18 months

K.2. Results

All test results were negative.

Appendix L. Summary of Peer Review Panel Comments

On May 22, 2014, the draft Technical Report on the toxicology and carcinogenesis studies of green tea extract received public review by the National Toxicology Program's Technical Report Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. C.R. Blystone, NIEHS, introduced the toxicology and carcinogenesis studies of green tea extract by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions, for the 2-year studies were *no evidence of carcinogenic activity* of green tea extract in male and female Wistar Han rats and in male B6C3F1/N mice, and *equivocal evidence of carcinogenic activity* of green tea extract in female B6C3F1/N mice

Dr. Carpenter noted receipt and distribution to the Panel of written comments from S.J. Smith on behalf of Taiyo Kagaku Co., LTD (Japan); Dr. T.L. Kurt on his own behalf; Dr. A.W. Wong, Dr. J.C. Griffiths, and H. Nguyen on behalf of the Council for Responsible Nutrition; M. McGuffin on behalf of the American Herbal Products Association; M. Blumenthal, Dr. S. Gafner, and A. Guevara on behalf of the American Botanical Council; and S.P. Mahinka on behalf of non-U.S. parties. He then recognized an oral public commenter, Dr. M. Zimmerman of the American Herbal Products Association, who spoke to the Panel by telephone.

Dr. Mirsalis, the first primary reviewer, noted the report accurately described and interpreted the findings of the 3-month and 2-year studies of green tea extract in rats and mice. He recommended reemphasizing in the report that as a result of the switch to Wistar Han rats, there are no appropriate historical data available for the current rat study. He stated the 1,000 mg/kg dose was too high, which could have been predicted from the effects seen in the 3-month study. Many of the findings in the report were only seen at that high dose. He asked why bilirubin was not measured when the test substance had previously demonstrated biliary toxicity and recommended the measurement should be included in the future. He speculated the accidental deaths in the 2-year rat study could be dose related and could be related to stress. He asked for clarification on the makeup of the green tea extract used in the studies, along with information on how it was prepared. He noted that every batch of green tea extract would be slightly different, and the material selected appeared to be representative of commercially available green tea extract. He asked how commonly nasal lesions are due to retrograde aspiration into the nasal cavity because he did not recall seeing these effects in other NTP studies. He recommended inclusion of a statement about when the studies were conducted, so the reviewer could assess the state of technology and science when evaluating the studies. He suggested removing references to "censoring" data on accidental deaths, as that is a politically charged term. Overall, he agreed with the proposed conclusions of the report.

Dr. Blystone, responding to Dr. Mirsalis' comments, said more context could be provided regarding the historical controls issue. He noted the 1,000 mg/kg dose was dose limiting and affected survival. Regarding the biliary toxicity, he said there were two markers of biliary toxicity that were assayed, and that was deemed sufficient. The accidental deaths were evaluated and deemed to be gavage accidents with fluid in the lungs. The table in the Discussion section

describing the constituents of green tea extract would be moved to the Materials and Methods section (Table 1). He agreed that retrograde aspiration is an uncommon occurrence, and could be discussed more in the report. He noted that the dates of the studies had been provided in the Materials and Methods section of the report. He said "censored" is actually a statistical term. Dr. G.E. Kissling, NIEHS, noted the term does have a specific statistical meaning. Dr. Mirsalis said he would defer to the term's statistical use.

Dr. Conner, the second primary reviewer, asked whether decreased survival of 1,000 mg/kg rats had an impact on data interpretation. Dr. Blystone said the low survival at the high dose could affect tumor formation response; however, no tumor response was seen at lower doses, so this did not affect the evaluation. Dr. Conner said the lesions underlying the equivocal evidence call for female mice were not sufficient and he would recommend a call of no evidence instead. Dr. Blystone responded that the rarity of the tongue tumors in female B6C3F1/N mice was the basis for the call of equivocal evidence. Dr. Conner also said there is often some degree of retrograde aspiration with compounds known to be irritants, and as rats are nose breathers, both incidental and treatment-related mortality are common. Dr. D.E. Malarkey, NIEHS, added that a retrospective study is underway to assess irritancy and reflux in the lung and nasopharynx.

Dr. Perdew, the third primary reviewer, addressed the reflux issue. He proposed that because green tea extract is quite viscous, some of the material may have remained on the needle as the gavage needle was being withdrawn. He recommended adding details to the report on how the extract was made. He advised that if the company involved is not willing to share the information, NTP should rethink buying from commercial sources. He asked to see full analyses performed on the green tea extract, particularly heavy metal analyses, perhaps in an appendix. He asked to see more information on the rationale for the doses selected, stating that citing literature is not an adequate rationale. He asked for an estimate of how many cups of green tea might be equivalent to the doses used. He said there should be more information in the report about the mutagenic potential of the components of green tea extract, which should be available in the literature.

Dr. Blystone said more details about the extract and its preparation could be added to the report; however, the available information is limited. NTP did measure the components of green tea extract. For the initial dose justification, the information available in the literature was used as the starting point and the National Cancer Institute provided some additional information. He agreed to emphasize that analyses were of the extract, not a beverage. He could provide some information about typical exposure to green tea extract, although that is beyond the purview of the report and enters into risk assessment. He agreed to review the information in the report regarding the mutagenicity of individual components of green tea extract. Referring to the Ames assay, Dr. Perdew asked what it would mean for the mutagenicity of green tea extract if individual components of green tea extract were not mutagenic. Dr. N.J. Walker, NIEHS, noted the extract contained more than the identified components, and this is typical of botanical extracts. Regarding whether to make or purchase the extracts, he said there is criticism attached to both methods of procurement. Dr. Perdew suggested that when a commercial acquisition is planned in the future, a deal should be made with the company to provide full information about the extract.

Dr. P.C. Howard, FDA, cautioned against making any statements of relevance to human consumption because that would force a risk assessment decision with inadequate information

within the study. Dr. Walker added that the testing was done on green tea extract, so it would be inappropriate to draw any comparisons with a green tea beverage. Dr. Perdew said perhaps he should have asked for inclusion of information about how the data would relate to the usual dose of green tea extract in humans. Dr. Howard reiterated that great care should be taken about relating these types of data to human consumption. Dr. Walker said in past Technical Reports on botanicals, simple statements relating to human consumption had been included, so this could be discussed for inclusion in this Technical Report.

Dr. Regan echoed Dr. Conner's remarks about reflux irritation in the nose. She asked if the study group had ever looked for inflammation in the animals' middle ears. Dr. A.E. Brix, Experimental Pathology Laboratories, Inc., said they had not. Dr. Conner agreed that irritation is often seen in eustachian tubes. Dr. Malarkey said if there was any inflammatory response at that location, it would have been noticed, but that site was not specifically examined. Dr. Regan said it was unlikely it would have been seen unless the area had been specifically examined. She also stated the incidence of two tumors of the tongue was not actual evidence of carcinogenicity. Dr. Mahrt agreed that the lesions of the nose may be related to retrograde aspiration.

Dr. Carpenter called for the draft conclusions to be projected.

Dr. Conner moved that the equivocal evidence conclusion be deleted for female mice, and the conclusion should be there is no evidence of carcinogenic activity at the various doses in both species and both sexes. Dr. Regan seconded the motion. Dr. Walker reminded the panel that the equivocal call had been made because squamous cell carcinoma of the tongue or oral cavity had never been seen before in historical control mice. Dr. Mirsalis said he was more comfortable with the equivocal call, based on the rarity of these neoplasms. Dr. Carpenter called for a vote on the motion. The motion carried, with 5 in favor and 1 opposed. Dr. Mirsalis explained his negative vote was due to the rarity of the tumors seen in two animals.

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