NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology and Carcinogenesis Studies of Ginseng (CAS No. 50647-08-0) in F344/N Rats and B6C3F1 Mice (Gavage Studies)

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF GINSENG

(CAS NO. 50647-08-0)

IN F344/N RATS AND B6C3F1 MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
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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, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

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

The 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.

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SUMMARY

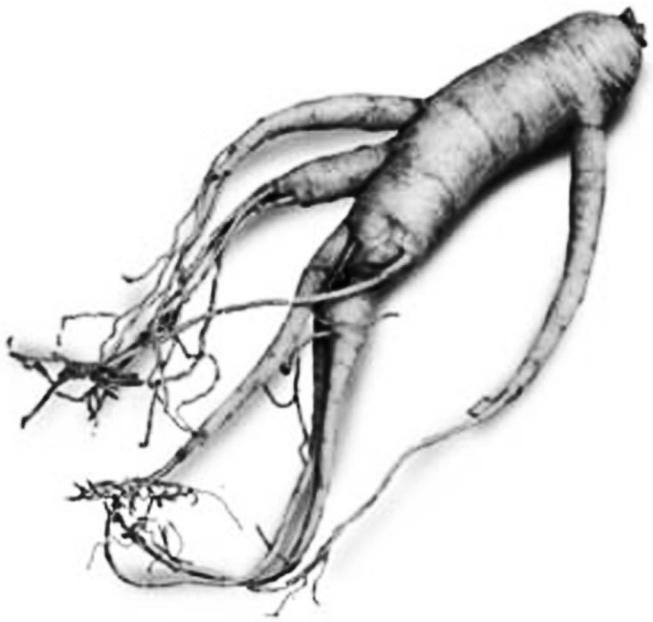
Background
Ginseng root is widely used as an herbal remedy and dietary supplement. We studied the effects of ginseng root extract on rats and mice to identify potential toxic or cancer-related hazards.

Methods
We administered solutions containing extracts of ginseng root in methylcellulose through a tube directly into the stomach of male and female rats and mice five times a week for two years. Groups of 50 animals received 1,250, 2,500, or 5,000 milligrams of ginseng root extract per kilogram of body weight. Similar groups of animals were given solutions of methylcellulose with no chemical added and served as the control groups. At the end of the study tissues from more than 40 sites were examined for every animal.

Results
Except for the female rats receiving 5,000 mg/kg of ginseng, survival and body weights of all exposed groups of animals were similar to their controls. There were no increases in the incidences of cancers at any sites, and the rates of mammary gland cancers in female rats were lower in animals given ginseng extract than in animals in their control group.

Conclusions
We conclude that ginseng did not cause cancer in male or female rats or mice. The incidence of mammary gland tumors in female rats was lower than the background rate in animals receiving ginseng.

ABSTRACT



GINSENG
CAS No. 50647-08-0

Synonyms: Ginseng; ginseng root extract; ginseng root neutral saponins; ginseng root tincture; ginsengwurzel extract; panax, *Panax ginseng; Panax schinseng;* prosapogenin
Botanical names: *Panax ginseng* C.A. Meyer; *Panax quinquefolius* L.
Trade name: Ginsana

CHEMICAL AND PHYSICAL PROPERTIES

Ginseng is a perennial aromatic herb widely used in herbal remedies, dietary supplements, cosmetics, and as a food additive. Ginseng was nominated for study by the National Cancer Institute based on significant human exposure through the uses described above and the lack of information on its toxicity. Male and female F344/N rats and B6C3F1 mice were administered extracts of ginseng root by gavage for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium, Escherichia coli,* and mouse peripheral blood erythrocytes.

2-WEEK STUDY IN RATS

Groups of five male and five female rats were administered ginseng in 0.5% aqueous methylcellulose by gavage at doses of 0, 125, 250, 500, 1,000, or 2,000 mg/kg, 5 days per week for 16 days. All rats survived to the end of the study. Mean body weight gain of 2,000 mg/kg males was significantly greater than that of the vehicle controls. There were no chemical-related gross or microscopic findings attributed to the administration of ginseng.

2-WEEK STUDY IN MICE

Groups of five male and five female mice were administered ginseng in 0.5% aqueous methylcellulose by gavage at doses of 0, 125, 250, 500, 1,000, or 2,000 mg/kg, 5 days per week for 17 days. All mice survived to the end of the study. The final mean body weight of 1,000 mg/kg males was significantly less than that of the vehicle controls. There were no significant chemical-related gross or histopathologic changes in dosed mice.

3-MONTH STUDY IN RATS

Groups of 10 male and 10 female rats were administered ginseng in sterile water by gavage at doses of 0, 1,000, 2,000, 3,000, 4,000, or 5,000 mg/kg, 5 days per week for 14 weeks. All rats survived to the end of the study. Mean body weights of all dosed groups were similar to those of the vehicle control groups. No lesions that were observed by gross or histopathologic examination were attributed to the administration of ginseng.

3-MONTH STUDY IN MICE

Groups of 10 male and 10 female mice were administered ginseng in sterile water by gavage at doses of 0, 1,000, 2,000, 3,000, 4,000, or 5,000 mg/kg, 5 days per week for 14 weeks. All mice survived to the end of the study. Mean body weights of all dosed groups were similar to those of the vehicle control groups. Although sporadic incidences of lesions were observed in the vehicle control and 5,000 mg/kg groups, there were no chemical-related gross or microscopic findings in dosed mice.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats were administered ginseng in sterile water by gavage at doses of 0, 1,250, 2,500, or 5,000 mg/kg, 5 days per week for 104 to 105 weeks. Survival of 5,000 mg/kg females was significantly less than that of the vehicle controls; however, the deaths were not attributed to the administration of ginseng because no histopathologic findings attributable to ginseng were found. Mean body weights of 5,000 mg/kg females were less than those of the vehicle controls after week 61 of the study, and mean body weights of other dosed groups of rats were similar to those of the vehicle controls throughout the study. No increases in the incidences of neoplasms or nonneoplastic lesions were attributed to the administration of ginseng. The incidence of mammary gland fibroadenoma was significantly decreased in 5,000 mg/kg females.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice were administered ginseng in sterile water by gavage at doses of 0, 1,250, 2,500, or 5,000 mg/kg, 5 days per week for 105 weeks. Survival of dosed groups was similar to that of the vehicle control groups. Mean body weights of dosed mice were similar to those of the vehicle controls. No neoplasms or nonneoplastic lesions were attributed to the administration of ginseng.

GENETIC TOXICOLOGY

Ginseng was not mutagenic in either of two independent bacterial mutagenicity assays, each conducted with or without exogenous metabolic activation enzymes. Bacterial strains tested included *S. typhimurium* strains TA97, TA98, TA100, TA102, TA104, and TA1535, as well as *E. coli* strain WP2 *uvrA/*pKM101. No significant increases were seen in the frequencies of micronucleated erythrocytes in the peripheral blood of male or female B6C3F1 mice exposed for 3 months to 1,000 to 5,000 mg/kg ginseng via gavage.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of ginseng in male or female F344/N rats or B6C3F1 mice administered 1,250, 2,500, or 5,000 mg/kg.

The incidence of mammary gland fibroadenoma was significantly decreased in 5,000 mg/kg female rats.

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

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in sterile water by gavage	0, 1,250, 2,500, or 5,000 mg/kg	0, 1,250, 2,500, or 5,000 mg/kg	0, 1,250, 2,500, or 5,000 mg/kg	0, 1,250, 2,500, or 5,000 mg/kg
Body weights	Dosed groups similar to the vehicle control group	5,000 mg/kg group 10% less than the vehicle control group after week 61	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group
Survival rates	30/50, 30/50, 37/50, 23/50	36/50, 27/50, 34/50, 24/50	32/50, 33/50, 38/50, 33/50	38/50, 31/50, 34/50, 32/50
Nonneoplastic effects	None	None	None	None
Neoplastic effects	None	None	None	None
Decreased incidences	None	Mammary gland: fibroadenoma (32/50, 30/50, 30/50, 16/50)	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Bacterial gene mutations		Negative in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, and TA1535 and in *Escherichia coli* WP2 *uvrA/*pKM101 with and without S9		
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:		Negative in both males and females		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on ginseng on November 19, 2009, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 19, 2009, the draft Technical Report on the toxicology and carcinogenesis studies of ginseng received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.S. Chhabra, NIEHS, representing NTP study scientist and lead author Dr. P.C. Chan, introduced the toxicology and carcinogenesis studies of ginseng by providing an overview of the NTP initiative on herbal products and dietary supplements. He described the uses of ginseng, the primary components of various ginseng products, and the design of the short- and long-term studies. The proposed conclusions from the 2-year studies were *no evidence of carcinogenic activity* of ginseng in male or female F344/N rats or B6C3F1 mice.

Dr. Sherley, the first principal reviewer, noted a treatment-associated change in estrous cycling that was not discussed in the text of the report. He questioned a statement that the deaths of some of the treated animals were not related to the chemical and suggested the statement be revised to reflect uncertainty about the cause of death.

Dr. Chhabra replied that an error in reporting the denominators of affected animals led to the impresssion of altered estrous cyclicity. With the correct data, no effect was noted. Regarding animal survival, Dr. Chhabra noted that no statistical significance occurred in survival rates and that there was no histopathologic diagnosis indicating cause of death.

Dr. Looney, the second principal reviewer, raised some generic questions about the statistical methods used in the studies, regarding power analyses, the use of Jonckheere's test, multiple comparisons, and the identification of outliers. He also suggested that median survival rather than mean survival might be a useful measure.

Dr. G.E. Kissling, NIEHS, replied that the study design using 50 animals per dose group has been used from the outset of the NTP program, and that an overall power analysis for a study with over 40 sites examined would be cumbersome. She noted that the Jonckheere's test was not an additional test, but rather was used as a decision-making test to determine which subsequent analysis was appropriate. Regarding analysis of various lesion types at a site, she stated that corrections for multiple testing were not applied. She noted that overall decisions for conclusions were not based solely on statistical criteria. Dr. Kissling said no outliers from the present study were included. She also noted that often, as in the present study, when survival is greater than 50% in a group, the median survival would be identical to the full study length.

Dr. Teeguarden, the third principal reviewer, thought the study was well conducted and agreed with the proposed conclusions. He and Dr. Nagarkatti asked for more detail about the source of the study material and how it was extracted from the base plant material. Dr. Chhabra replied that uncertainty about sources and strength of materials are commonplace with herbal products. In the present study, the several preparations of ginseng materials yielded consistent profiles of the component ginsenosides and thus were considered fairly standard.

Drs. Eastmond, Sherley, and Pino all mentioned that in various draft reports there was some inconsistency in how decreased incidences of tumors were presented in the conclusion statements. Dr. D.E. Malarkey, NIEHS, noted that the studies were designed primarily to detect increases in tumor incidence and that level of evidence conclusions were not assigned to decreases. Dr. M.J. Hooth, NIEHS, added that factors such as historical background rates and concurrent decreases in body weight were also taken into account in considering whether decreased incidences could be associated with treatment. Dr. Malarkey added that decreased tumor incidences in studies such as these should not be interpreted as evidence of protective effects.

Dr. Sherley moved, and Dr. Portier seconded, that the conclusions be accepted as written. The motion was approved with six yes votes and four no votes. Drs. Eastmond, Teeguarden, Portier, and Pino voted no; they thought the decrease in fibroadenoma should be clarified that it may or may not be related to ginseng administration.

INTRODUCTION

GINSENG

CAS No. 50647-08-0

Synonyms: Ginseng; ginseng root extract; ginseng root neutral saponins; ginseng root tincture; ginsengwurzel extract; panax; *Panax ginseng*; *Panax schinseng*, prosapogenin

Botanical names: *Panax ginseng* C.A. Meyer and *Panax quinquefolius* L.

Trade name: Ginsana

CHEMICAL AND PHYSICAL PROPERTIES

Ginseng is a perennial aromatic herb with a short underground stem (rhizome) associated with a fleshy white root. Its root system consists of the primary root, its branches, and adventitious roots developed from the rhizome. The above-ground part of the plant is a 30 to 70 cm single stem that is replaced annually (Sticher, 1998). Plants bloom after 2 years of growth, reach maturity in 5 years, and can be harvested in 6 years (Hook, 1979). True ginsengs are members of the genus *Panax* in the *Araliaceae* family. In addition to *Panax ginseng* and *Panax quinquefolius*, other ginsengs include *Panax japonicus* (Japanese ginseng), *Panax notoginseng* (Sanqui or Tienqi ginseng), *Panax elegantior* (Pearl ginseng), *Panax* pseudoginseng (Himalayan ginseng), and *Panax zingiberensis* (ginger ginseng) (Ocollura, 1997).

Some plants are not true ginsengs (i.e., different genus or family), but they have the term ginseng in their common names. These include Siberian ginseng *(Eleutherococcus senticosus),* which is widely used in dietary supplement preparations, Prince ginseng *(Pseudostellaria heterophylla),* Indian ginseng (Ashwangdha), and Brazilian ginseng *(Pfaffia paniculata)* (Ocollura, 1997). Except where it is impossible to distinguish the form of ginseng, these products are not discussed further. In the present text, the term ginseng refers to *Panax ginseng* and *Panax quinquefolius*, the two most popular species. Their profiles of ginsenosides are similar with some differences in minor ginsenosides (Li *et al.*, 2000). When the other species are discussed, their specific names are used.

With the use of high performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry (LC/MS), several classes of compounds have been identified from ginseng root. These include triterpene saponins, essential oil-containing polyacetylenes and sesquiterpenes, polysaccharides, peptidoglycans, nitrogen-containing compounds, and various ubiquitous compounds such as fatty acids, carbohydrates, and phenolic compounds (Sticher, 1998).

The chemical constituents of ginseng believed to contribute to its pharmacological effects are triterpene saponins or steroid saponins. These compounds are named ginsenosides Rx according to their mobility on thin-layer chromatography plates, with the polarity indicator (x) decreasing from index letters "a" to "h." This property is a function of the number of monosaccharide residues in the sugar chain. Ginsenosides are glycosides containing an aglycone (protopanaxadiol or protopanaxatriol) with a dammarane skeleton. More than 40 ginsenosides have been isolated from ginseng roots and categorized into three groups by their aglycones: protopanaxadiol-ginsenosides, protopanaxatriol-ginsenosides, and oleanolic acid-saponins (Sticher, 1998; Chang *et al*., 2003; Fuzzati, 2004).

Almost all dammarane ginsenosides isolated from white ginseng root are derivatives of 20(S) protopanaxadiol and 20(S) protopanaxatriol, and almost all can also be found in red ginseng. However, some ginsenosides (20R Rg2, 20R Rh1, Rh2, Rs1, Rs2, Q-R1, and NG-R1) are characteristic saponins for red ginseng. The 20R compounds are degradation products formed by heating and hydrolysis during steaming (Sticher, 1998). Asian ginseng contains ginsenoside Rf, which is not found in American ginseng (Lang *et al*., 1993). The structures of the more common ginsenosides are shown in Figure 1 (Gillis, 1997; Sticher, 1998).

The purity of each ginsenoside can be determined by melting point (Fisher-John Apparatus), optical rotations (Jasco DIP-370 Instrument), and positive fast atom bombardment mass spectroscopy (FAB/MS)(VG-VSEQ, tupe EbqQ) (Lee *et al*., 1996).

Panax ginseng, Panax quinquefolius, and *Panax notoginseng* are closely related taxonomically and have similar chemical compositions. Generally, they contain total ginseng saponin below 0.1%, and the saponins constitute primarily dammarane-type triterpenes, with a higher content of panaxadiol and panaxatriol and a very low content of oleanolic acid as sapogenin. *Panax notoginseng* contains no oleanolic acid sapogenin. The total saponin content of the remaining *Panax* species is 10% to 20%, and oleanolic acid is the major sapogenin (Peigen, 1989).

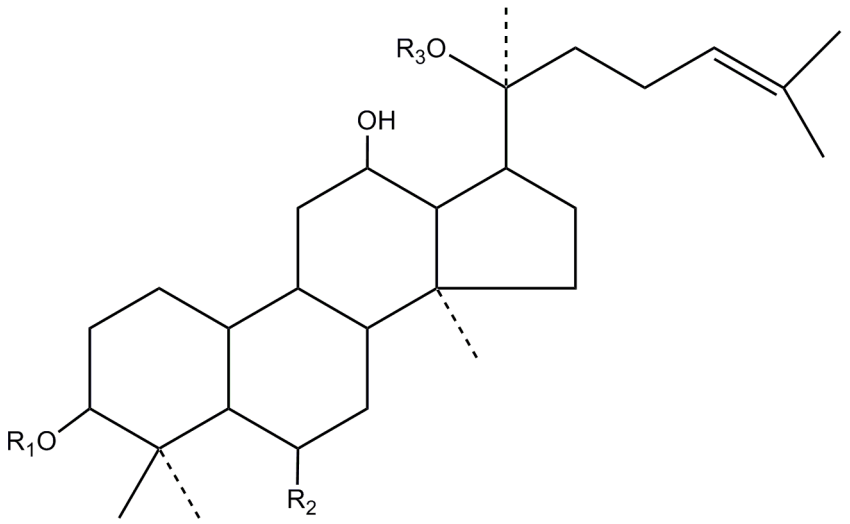
The stems, leaves, flowers, flower buds, and fruits contain more ginseng saponins than the ginseng root. The underground part contains higher amounts of ginsenosides Rb1, Rc, and Rg1, while the above-ground parts contain higher amounts of ginsenosides Rd, Re, and Rg1 (Peigen, 1989).

PRODUCTION, USE, AND HUMAN EXPOSURE

Several *Panax* species are indigenous to the Northern Hemisphere, from the eastern Himalayas through China and Japan to North America. *Panax quinquefolius* is found on rich, rocky, shaded, cool slopes of eastern North America, from Quebec to Manitoba and south to northern Florida, Alabama, and Oklahoma. Its peak abundance is in the Cumberland Gap region of southern Appalachia. Wild ginseng is now considered a threatened, rare, or endangered species in many areas due to overzealous harvest of the root for commercial purposes. Because of continual harvest and use over thousands of years, the natural supply of ginseng root was exhausted in China long ago (Eastman, 1976; Lewis and Zenger, 1982; Sticher, 1998).

When American ginseng was initially exported in the early eighteenth century, wild *Panax ginseng* had already become extremely scarce in China. The relative abundance and quality of wild American ginseng opened the way for development of cultivated American ginseng as an export crop in the twentieth century (Hsu, 1979). Although wild-harvested root is still a United States export, ginseng is now cultivated in China, Korea, Japan, and North America. Ginseng is an especially important crop in the state of Wisconsin (Hsu, 1998; Sticher, 1998).

Despite the growing market for extracts and powders, the most popular ginseng products remain the white and red roots. White ginseng is prepared by removing the small, hairy roots, scraping the outside skin off the main root, and then drying in the sun, over charcoal, or in an oven. Red ginseng is prepared by removing all soil from the root, cutting off the hairy and branch roots, and brushing the skin until the root looks white; the root is then steamed 3 hours, dehydrated in a dry room, and dried in the sun (Hook, 1979). Ginseng roots may be graded by size. For example, grades of Korean ginseng are Heaven 15 and Heaven 30, which means it takes 15 or 30 roots, respectively, to fill a standard ginseng



Ginsenoside	R1	R2	R3
20(S)-protopanaxadiols			
Rb1	glucose-glucose	Hydrogen	glucose-glucose
Rb2	glucose-glucose	Hydrogen	glucose-arabinose (pyranose form)
Rc	glucose-glucose	Hydrogen	glucose-arabinose (furanose form)
Rd	glucose-glucose	Hydrogen	glucose
20(S)-protopanaxatriols			
Re	Hydrogen	-O-glucose-rhamnose	Glucose
Rf	Hydrogen	-O-glucose-glucose	Hydrogen
Rg1	Hydrogen	-O-glucose	Glucose
Rg2	Hydrogen	-O-glucose-rhamnose	Hydrogen
Rh1	Hydrogen	-O-glucose	Hydrogen

FIGURE 1
Structures of Common Ginsenosides
Gillis (1997) and Sticher (1998)

container; the larger the number, the smaller and less valuable the root (Ocollura, 1997). Cultivated ginseng is propagated from seeds harvested from ripe fruits of 4- to 5-year-old plants; germination occurs in 18 to 20 months. Seedlings may be transplanted to permanent beds when they are 1 or 2 years old. Because wind, rain, and direct sunlight can be harmful, the plants are grown within artificial shelters. Four to 6 years later, the root is harvested between August and October when the above ground portion turns yellow (Sticher, 1998).

Because ginseng is expensive to produce, adulteration or substitution with cheaper products occurs (Ocollura, 1997). Some products sold as ginseng contain *Mandragora officinarum*, with hyoscine; *Rauwolfia serpentina*, with reserpine; and Cola, with caffeine. Some products were adulterated with phenylbutazone and aminopyrine (Chandler, 1988). To protect its interests in the Hong Kong market, the Ginseng Board of Wisconsin organized a labeling system for genuine American ginseng products (Proctor, 1996). The American Botanical Council initiated a comprehensive ginseng evaluation program for hundreds of commercial ginseng products to detect adulteration (American Botanical Council, 2001). Currently, the ginseng product that has been standardized and used in research studies is Ginsana™ or G115. Ginsana™ is an extract of *Panax ginseng* C.A. Meyer containing 4% ginsenosides and is produced by Pharmaton Limited (Lugano-Bioggio, Switzerland). The preparation, though standardized, is a mixture of many chemical entities.

Worldwide, ginseng is the most popular herbal medicine (Blumenthal, 2001, 2002; Barnes *et al*., 2008). Ginseng production was a 3 billion dollar industry with production estimated to be 22.2 million pounds in 1993. South Korea and China each produced about 10 million pounds; the United States produced at least 1.4 million pounds; Japan produced 76,000 pounds; and Canada produced 694,000 pounds. Much of the North American ginseng is marketed directly from the farm to ginseng brokers in Hong Kong, the major importer, distributor, processor, and retailer of ginseng. Over 80% of ginseng grown in North America is shipped to the Hong Kong market as is much of the ginseng from China and Korea (Proctor, 1996).

Redistribution of ginseng from Hong Kong is worldwide with major destinations being Taiwan, Japan, Malaysia, Singapore, and the United States. There has also been a strong European market for ginseng since the 1960s with well-established markets in Scandinavia, Poland, Germany, Spain, Holland, Belgium, the United Kingdom, France, and Italy.

Many Canadian and United States ginseng growers and licensed wild ginseng dealers sell their products directly to consumers. Most products are small, medium, or large roots, either fresh or dried. Some have also expanded their product lines to include capsules, powder, extracts, root slices, tea bags, candy, lotions, and soaps (Carl, 1997; Hsu, 1998; Woods Grown Naturally Canadian Ginseng, 1998). According to data from Information Resources Inc., sales of ginseng in 2008 were over 8 million dollars in the United States (Cavaliere *et al*., 2009).

There are differences in the composition of the various ginseng species, the manner of sample preparation, and the age and part of the plant extract. These differences account for the varying effects reported; some of the saponins produce effects directly opposite to those produced by others. Today, purification and quantitation of ginsenosides can be conducted by conventional HPLC, electrospray HPLC, and MS. The more highly regarded ginseng products have been shown to contain more ginsenosides of the protopanaxatriol type. But proper identification and labeling of ginseng products are lacking as quality control of ginseng products on the market is not required**.** G115 or Ginsana™ is the only ginseng product that has been standardized to contain 4% ginsenosides, but the proportions of the different ginsenosides cannot be guaranteed.

Ginseng is a popular herbal remedy purported to enhance stamina and endurance for both mental and physical performance, especially under stress (anti-stress). Ginseng products are one of a spectrum of approaches referred to as complementary and alternative medicines (CAMs), agents that have claims to prevent or treat disease (Barnes *et al*., 2008). In general, CAMs, including ginseng, have insufficient evidence that they are safe and effective. Many of the reports are from non-peer-reviewed literature and lack appropriate, controlled scientific evaluation. In the United States, ginseng is referred to as a "nutraceutical," a food extract claimed to have human medicinal effects. The root is sold directly as an herbal remedy or it may be extracted or powdered for use in dietary supplements. Ginseng is also added to foods, cosmetics, and beverages. The major share of the nutraceutical market consists of products sold as dietary or nutritional supplements in health food stores, pharmacies, supermarkets, and mail-order houses. Because ginseng products are sold as dietary supplements and are not subject to regulatory requirements, the composition of preparations is variable.

Herbalists classify ginseng as an "adaptogen," defined as a natural herb product with antioxidant activity that

increases the body's resistance to stress, trauma, anxiety, and fatigue. Adaptogens are thought to act with nonspecific activity on the body according to the body's needs. Beneficial effects claimed for ginseng include antiaging and it is used to combat diabetes, radiation sickness, psychiatric disorders, hypercholesterolemia, cancer, and ulcers (Chandler, 1988; Kitts and Hu, 2000; Helms, 2004). Ginseng is purportedly useful for cardioprotection, vasorelaxation, anemia, atherosclerosis, hypertension, and edema, and may have beneficial effects on the central nervous system with improved memory, learning, and behavior (Gillis, 1997; Scimone and Scimone, 1998; Wang and Lee, 1998). Ginseng reportedly has pharmacological activity that may increase alertness, improve concentration, increase resting oxygen uptake and transport, abrogate gastrointestinal disorders, and relieve depression (Gillis, 1997). Ginseng may have immune-boosting activity and is used in the hope of preventing and treating colds and influenzas and reducing stress (Gillis, 1997; Scimone and Scimone, 1998). Ginseng is also used as an analeptic, tonic, stomach pain analgesic, and aphrodisiac (Chang *et al*., 1986). Evidence from *in vitro* and *in vivo* studies suggests that ginseng is an antioxidant and stimulates nitric oxide release (Kim *et al.*, 1992; Kang *et al*., 1995; Maffe *et al*., 1999). The reported variable effects of ginseng may be due to the different quantities and types of saponins in the products. At times, opposite effects have been reported; for example, hypertensive and hypotensive effects, histamine and antihistamine-like actions, and stimulatory or depressant activity on the central nervous system (Chong and Oberholzer, 1988).

There is a potential for widespread exposure to ginseng because of its use as an herbal remedy, its presence in dietary supplements and cosmetics, and its use as a food additive. Before the boom in the herbal supplement industry, an estimated 5 to 6 million people in the United States were using ginseng regularly (Chandler, 1988). Information Resources, Inc. (2008), reported sales in the United States of all herbal supplements, generated from the food, drug, and mass market channels only (15% of total channels), of almost 290 million dollars; of these, ginseng was ranked ninth in sales with over 8 million dollars. In 2007, the Centers for Disease Control and Prevention conducted a National Health Interview Survey to measure the United States population's use of CAMs (Barnes *et al*., 2008). After surveying nearly 30,000 families, it was estimated that one in nine children and four in 10 adults in the United States used complementary and alternative medicine therapy in the 12-month period prior to the survey; 14.1% of these adults had used ginseng. Extrapolating from these data, it was estimated that 19,000 children and 3.4 million adults in the United States had used ginseng in 2007 for health reasons (Barnes *et al.*, 2008).

A potential for occupational exposure to ginseng exists, especially in the bulk packaging and processing of dietary supplements containing ginseng extracts, powders, and concentrates. Exposure to ginseng from agricultural practices would be expected to be minimal since only the plants and plant roots are handled.

No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace maximum allowable levels of ginseng. Ginseng was not included in the American Conference of Governmental Industrial Hygienists list of compounds for which recommendations for a Threshold Limit Value (TLV) or Biological Exposure Index (BEI) are made (ACGIH, 2009) nor in the National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 (NIOSH, 1990).

REGULATORY STATUS

Since 1994, dietary supplements have been regulated in the United States under the Dietary Supplement Health and Education Act (DSHEA); most ginseng products are covered under the DSHEA, but their content is not regulated. The DSHEA requires no proof of safety for dietary supplements on the market prior to October 15, 1994. Labeling requirements for such supplements allow warnings and dosage recommendations as well as substantiated "structure or function" claims. All claims must prominently note that they have not been evaluated by the Food and Drug Administration, and they must bear the statement "This product is not intended to diagnose, treat, cure, or prevent any disease" (Croom and Walker, 1995).

Ginseng is more closely regulated in Europe. Currently, the German government's Commission E allows *Panax ginseng* products containing at least 1.5% ginsenosides, calculated as ginsenoside Rg1, to be labeled for use as a tonic for invigoration and fortification during times of fatigue and debility, for declining work capacity and concentration, as well as during convalescence (Blumenthal *et al*., 1996). Ginseng is also specified in the Swiss, Austrian, and French pharmacopeias (Sticher, 1998). The Swiss pharmacopeia requires a total ginsenoside content of not less than 2.0%, calculated as ginsenoside Rg1.

International trade in American ginseng is regulated under the provisions of the Convention on International Trade in Endangered Species (CITES), which regulates

trade through permit requirements for imports, exports, and re-exports of listed species. American ginseng is listed in CITES, Appendix II, controlling and monitoring its trade "in order to avoid utilization incompatible with survival" (Singer, 1979). Harvest and commerce are regulated and restricted jointly and separately by state agencies, the United States Fish and Wildlife Service, and the United States Department of Agriculture (Foster, 1996).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The degradation and metabolism of ginsenosides has been studied in animals and *in vitro* using acids, enzymes, and intestinal bacteria (Han *et al*., 1982; Odani *et al*., 1983; Strömbom *et al*., 1985; Karikura *et al*., 1990; Hasegawa *et al*., 1996; Akao *et al*., 1998). After oral administration, the protopanaxatriol ginsenosides (Rg1) are hydrolyzed to ginsenoside Rh1 and its hydrated form under mild acidic conditions similar to gastric fluid. Protopanaxadiol ginsenosides (Rb1) are metabolized to M1 [20-*O*-*β*-D-glucopyranosyl-20(*S*)-protopanaxadiol] or compound K in rats and humans by intestinal anaerobes via stepwise cleavage of the sugar moieties (Hasegawa *et al*., 1996). Hasegawa *et al.* (1997) identified *Prevotella oris* strains of intestinal bacteria that hydrolyze Rb1. Ota *et al.* (1991) reported that protopanaxadiol is formed from Rh2 as a result of deglycosylation by B16 melanoma cells *in vitro*. The metabolic process led to growth inhibition of the B16 cells.

The absorption of Rb1 from the intestine of rats was low (Odani *et al*., 1983). In mice, after an oral dose of Rb1 or M1, a metabolite of protopanaxadiols, the M1 level in the serum gradually increased, peaked at 8 hours after oral administration of Rb1, and decreased with time; intact Rb1 was not detected in the serum (Wakabayashi *et al*., 1997). Rg1 was rapidly absorbed (30% after 1 hour) and metabolized by mice after oral administration. Mouse urine and feces contained little unchanged Rg1 but did contain high levels of metabolites including Rh1 and 25-OH-Rh1 (Sticher, 1998).

By measuring the total amount of protopanaxatriol and protopanaxadiol ginsenosides as aglycones in human urine, Cui *et al*. (1997) demonstrated that about 1.2% of the orally ingested dose of protopanaxatriol ginsenosides (3 mg) and 0.2% of the protopanaxadiol ginsenosides (7 mg) can be recovered. However, neither the individual ginsenosides nor their metabolites were identified. Compound K, which is the main intestinal bacterial metabolite of protopanaxadiol ginsenosides, was identified in human serum 8 hours following oral administration of ginseng (Shibata, 2001). Tawab *et al*. (2003) showed that following oral administration of ginseng in humans, the ginsenosides and two hydrolysis products of the protopanaxatriol ginsenosides, G-Rh1 and G-F1, reached the systemic circulation. In addition, Compound K was detected in plasma and urine.

Ginsenoside Rg1 (protopanaxatriol) showed an extremely short half-life of 27 minutes after intravenous administration to mini-pigs. In contrast, the protopanaxadiol ginsenoside Rb1 showed a half-life in the β-phase of 16 hours (Sticher, 1998).

The activities of CYP2A6, 2C9, 2C19, and 2D6 were not altered by ginseng extract or individual ginsenosides up to 10 μM (Etheridge *et al*., 2007). However, the activity of CYP2E1 was reduced by ginsenosides F1 and Rh1 and not by the ginseng extract. The activity of CYP3A4 was moderately reduced by both ginseng extract and ginsenosides F1 and Rh1. Ginseng extract and Rh1 modestly stimulated Pgp ATPase activity in a concentration dependent manner. These data suggest that the clearance of a variety of drugs may be diminished by concomitant use of ginseng herbal supplements due to the inhibition of CYP450 enzymes but less so by Pgp-mediated effects.

Chang *et al.* (2007) demonstrated that ginsenosides Compound K and Rg1 elicit potent enhancing and suppressing effects, respectively, on glucose uptake across human intestinal Caco-2 monolayer through modulation of Na+/glucose cotransporter 1 (SGLT1) expression.

TOXICITY

Experimental Animals

The acute toxicity values listed in the Registry of Toxic Effects of Chemical Substances (1998) for various ginseng products are shown in Table 1.

Male and female Sprague-Dawley rats fed diets containing G115 (Ginsana™) at up to 15 mg/kg per day for 13 weeks developed no histopathologic changes (Hess *et al*., 1982). No toxic effects were noted in rats following ingestion of ginseng extract at daily doses of 105 to 210 mg/kg for 25 weeks (Popov and Goldwag, 1973); no details on this study were available.

In a chronic study in mice, no significant differences in mean weights or survival were observed in mice consuming *Panax ginseng* even though increased behavioral responses to mild stress were noted (Bittles *et al*.,

TABLE 1
LD_{50} Values for Ginseng

Compound	Species	Route	LD_{50} (mg/kg)
Panax ginseng	Rat	Oral	750
	Mouse	Oral	200
	Mouse	Intraperitoneal	54
Ginseng root extract	Mouse	Intraperitoneal	545
Ginsenoside No. 3	Mouse	Intraperitoneal	910
Ginseng, saponin extract	Mouse	Intraperitoneal	637

1979). There were three groups with 90 animals per group. One group consumed ginseng extract from 8 weeks of age throughout life. The second group received ginseng from 52 weeks onward, and the third group served as untreated controls. Ginseng extract was administered in drinking water at a dose of 8 mg/kg per day, corresponding to 40 mg of whole root/kg per day, which was considered low. No attempt to define the maximum tolerated dose was made. Ginseng administration did not alter the lifespan of the mice, but their behavioral response to stress was exaggerated.

No evidence of toxicity was observed in groups of four male and four female beagle dogs fed diets containing 0, 1.5, 5, or 15 mg ginseng extract G115/kg body weight per day for 3 months (Hess *et al*., 1983). Although several significant differences in clinical chemistry and hematology values were noted, no consistent dose-response relationship occurred and all values were within normal physiological ranges for beagle dogs. Gross and microscopic examinations of major organs revealed no morphology or pathologic effects. The highest dose, 15 mg/kg, is approximately twice the recommended dose for humans.

Humans

Toxicity of ginseng in humans is difficult to evaluate; when toxicity is reported, the form, quantity, and quality of the ginseng used and the diet, use of other drugs, and/or stress conditions of the patients are not known. Typical dosages are up to four 500 or 600 mg capsules per day. The dosage drops to 100 mg one or two times per day for products standardized to contain 5% to 7% ginsenosides. Some herbalists advocate using ginseng for 3 weeks, followed by a 1-week break. Users are cautioned not to exceed recommended dosages and to discontinue use if ginseng raises blood pressure or produces hot flashes, insomnia, nervousness, or irritability (McGuffin *et al.,* 1997, Blumenthal *et al*., 1996).

Characteristic signs and symptoms of overexposure to ginseng have been named ginseng abuse syndrome. Siegel (1979) described suspected ginseng abuse syndrome in 133 humans using ginseng regularly for at least 1 month. The doses varied from 8 to 10 g three times per day for capsules, 0.5 to 3 g twice per day for roots, 1 to 2 g three times per day for ground powders, and 2.5 to 5 mL per day for extracts. Most subjects experienced central nervous system excitation and arousal. Fourteen subjects purportedly experienced hypertension, nervousness, sleeplessness, skin eruptions, and morning diarrhea; five had edema; and 10 became euphoric, restless, agitated, and insomniac. Ginseng abuse syndrome appeared periodically in the first 12 months of ginseng use, but the syndrome was rarely reported in follow-up examinations at 18 and 24 months.

There are individual reports of possible adverse effects including mastalgia with diffuse mammary nodularity and vaginal bleeding in postmenopausal women (Chandler, 1988); an episode of postmenopausal bleeding (Hopkins *et al*., 1988), hypertension, dizziness, and inability to concentrate (Hammond and Whitworth, 1981), and headache, nausea and vomiting, and chest tightness after ingesting a large quantity of ethanol-extracted ginseng (Ryu and Chien, 1995).

The FDA's Special Nutritionals Adverse Event Monitoring System reported 114 illnesses or injuries associated with the use of special nutritional products and dietary supplements containing ginseng as of May 14, 1998 (FDA, 1998). Thirteen deaths were reported. The following effects were reported for 17 products apparently containing only ginseng as an active ingredient: seizures, mild strokes, pruritus, jaundice, vomiting, nausea, diarrhea, perspiration, dermatomyositis, coma, stomach pains, rash, heart palpitations, sweating, dizziness, blurred vision, and abnormal uterine bleeding.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Sprague-Dawley rats were fed a diet containing G115 at up to 15 mg/kg per day for two generations (Hess *et al*., 1982). No treatment-related histopathologic changes were observed in the F_1 rats. Treatment related gross changes were not observed in the F_0 or F_2 animals.

Chan *et al*. (2004) studied embryotoxicity of ginsenoside Rc and Re in *in vitro* rat whole embryo culture and found that ginsenoside Re resulted in a significantly lower median morphological score, a decreased number of somites, smaller yolk sac diameter, and shorter crown-rump length. In contrast, there were no significant embryotoxic effects by exposure to ginsenoside Rc. These results show evidence of significant variability in the embryotoxic effects of different ginsenosides.

Liu *et al.* (2005) employed whole embryo culture to explore the developmental toxicity of ginsenoside Rb1 (GRb1) on mouse embryos. The results suggest that GRb1 exhibited a teratogenic effect during the mouse organogenetic period. Further study demonstrated that ginsenoside GRg1 exerted embryotoxicity during both rat and mouse organogenetic periods, with the effect higher in rats than mice (Liu *et al*., 2006). These authors suggest that, before more data in humans are available, ginseng should be used with caution by pregnant women in their first trimester.

Humans

No information related to the reproductive or developmental toxicity of ginseng in humans was found in the literature.

CARCINOGENICITY

Experimental Animals

No chronic carcinogenicity studies of ginseng or ginsenosides were identified in the literature.

Humans

No epidemiology studies or case reports investigating the association of exposure to ginseng and cancer risks in humans were identified in the literature.

American ginseng induced the expression of the estrogen regulated genes. Rb1 ginsenoside was shown to be responsible for the induction of estrogen-related genes in the estrogen receptor positive breast cancer cell line MCF-7 (Taback *et al*., 1996). The expression of pS2 induced by ginseng and Rb1 was inhibited by tamoxifen. These results support the evidence suggestive of a steroidal effect for ginseng seen in some human case studies.

GENETIC TOXICITY

The published data from genotoxicity studies with ginseng or ginsenosides indicate a lack of mutagenicity, and in fact, a number of publications suggest that ginseng and related ginsenosides may possess significant antimutagenic activity, reducing the effects of known mutagens in both *in vitro* and *in vivo* test systems. Neither a water extract of 3-year-old *Panax quinquefolius* roots (not standardized, and tested at concentrations up to 36 mg/mL) nor a 1-butanol extract (also tested up to 36 mg/mL) containing ginsenosides was mutagenic in a forward mutation assay using *Salmonella typhimurium* strain TM 677 (*uvr*B, rfa, pkM101, gal-, bio-, his+), with or without metabolic activation (Chang *et al*., 1986). Aqueous solutions of dried powders of *Panax japonicum* and *Panax ginseng* (100 mg/mL) did not show differential toxicity in DNA damage sensitive strains of *Bacillus subtillis* (H17Rec+ and M45Rec-), and they were not mutagenic in the standard *S. typhimurium* tester strains TA98 and TA100, with or without PCB-induced rat liver S9 (Morimoto *et al*., 1981). In cultured Chinese hamster V79 cells, a root extract of *Panax ginseng* (0 to 1 mg/mL) was reported to inhibit DNA synthesis, measured by [^{3}H]thymidine incorporation, but to increase the rate of DNA excision repair processes following treatment of cells with ultraviolet radiation or methyl methanesulfonate (Rhee *et al*., 1991).

Two ginsenosides identified as active components of ginseng, Rb1 and Rg1, were screened for antimutagenic activity against a known direct-acting mutagen [2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide] in *S. typhimurium* strain TA100; both Rg1 and Rb1 reduced the activity of the mutagenic agent (Ohtsuka *et al*., 1995). A decrease in *hgprt* mutations induced by methyl methanesulfonate was observed in Chinese hamster V79 cells treated with *Panax ginseng* root extract (Rhee *et al*., 1991). A well-characterized, standardized extract of *Panax ginseng* containing more than 3% of the ginsenoside Rg3 *(Phoenix ginseng)* was effective at reducing the frequencies of chromosomal aberrations and micronuclei in bone marrow cells of Swiss albino mice treated with 7,12-dimethylbenz[a]anthracene (DMBA) or croton oil (Panwar *et al.*, 2005a). American ginseng extract

(Canadian Phytopharmaceutical Corporation) containing 10.1% ginsenosides (Rg1, Re, Rb1, Rc, Rb2, and Rd), 2% additional ginsenosides (made up of F11, Ro, isomers of Rd, and traces of malonyl ginsenosides) was reported to reduce ulcerative colitis-associated DNA damage in mouse colon epithelial cells measured by the Comet assay (Jin *et al*., 2008). Additional evidence of antimutagenic activity of ginsenosides or uncharacterized extracts of *Panax ginseng* derives from a number of recent studies involving co-administration or sequential treatments *in vitro* or *in vivo* with known mutagens such as cyclophosphamide (Zhang *et al*., 2008), mitomycin C (Pawar *et al*., 2007), ultraviolet light (Jeong *et al*., 2007), and gamma radiation (Ivanova *et al*., 2006).

STUDY RATIONALE

Ginseng and ginsenosides were nominated for study by the National Cancer Institute based on significant human exposure through their use in herbal remedies, dietary supplements, cosmetics, and food additives, and the lack of information on their toxicity.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Ginseng

Ginseng extract from the plant *Panax ginseng* C.A. Meyer was obtained from Extracts Plus, Inc. (Vista, CA), in two lots (3021261 and 302500702) and from Plus Pharma, Inc. (Vista, CA), in one lot (3031978). Lot 3021261 was used during the 2-week studies, lot 302500702 was used during the 3-month studies, and lot 3031978 was used during the 2-year studies. All lots were produced by extracting ginseng root with 80% aqueous ethanol. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Laboratories, Chemistry Support Services (Columbus, OH), and the study laboratory at Battelle Columbus Operations (Columbus, OH) (Appendix I). Multiple standardized analyses of all three lots were performed by Covance Laboratories, Inc. (Madison, WI), and proton-induced X-ray emission (PIXE) spectroscopy was performed on each lot by Element Analysis Corporation (Lexington, KY). Reports on analyses performed in support of the ginseng studies are on file at the National Institute of Environmental Health Sciences.

Infrared spectrometry was used to obtain benchmark fingerprints for each lot, and spectra were compared between lots; spectra were similar for all lots. All lots of the chemical, a light tan powder, were characterized as ginseng by weight loss on drying; analyses for vitamin, total protein, ash, total fatty acid, total fat, total dietary fiber, amino acid, chloride, metal, organophosphate pesticide, and nitrosamine content; PIXE analysis for common elements other than carbon, hydrogen, and oxygen; ion chromatography for quantitation of selected anions; total carbohydrate and ethanol-soluble carbohydrate analysis; high-performance liquid chromatography (HPLC) with refractive index detection for mono- and disaccharide analysis and quantitation of selected sugars; and size exclusion chromatography to determine the molecular weight distribution of the glycans.

The purity of each lot of ginseng was determined by the analytical chemistry laboratory based on the profile of ginsenosides in the test material using methodologies based on the *American Botanical Council* (2001), which was a comprehensive study of ginseng products. Solutions of *Panax ginseng* C.A. Meyer were analyzed and compared to authentic standards of available ginsenosides using HPLC.

One HPLC system was optimized for the separation of the ginsenosides and a second system was optimized to obtain the overall purity profile of the test material. Weight percentages of the ginsenosides in the test material were determined using the method of standard addition and analysis by HPLC. Total ginsenosides were determined to be 7.4% for lot 3021261, 10.9% for lot 302500702, and 7.4% for lot 3031978.

Additional components of the test material were characterized using reverse phase HPLC with mass spectrometric (MS) detection and positive electrospray ionization to confirm the identity of ginsenosides and gas chromatographic analyses with flame ionization and/or MS detection to identify six, 13, or 12 non-ginsenoside components of lots 3021261, 302500702, and 3031978, respectively.

Taken together, these data indicate that all three lots of the test material were ginseng and their composition was consistent with the expected composition of typical ginseng.

Stability studies of lot 3021261 of the bulk chemical were performed using HPLC. These studies indicated that ginseng was stable as a bulk chemical for at least 14 days when stored protected from light in sealed glass containers at temperatures up to 25° C. At 60° C, some physical changes to the sample were evident as well as slight changes in the chromatographic profile of three of the individual ginsenosides. To ensure stability, lots 3021261 and 302500702 were stored at room temperature in sealed amber glass bottles containing a headspace of argon gas, and lot 3031978 was stored at room temperature in double plastic bags under an argon headspace, sealed with tape, inside sealed plastic drums also containing an argon headspace. Periodic reanalyses of the bulk chemical were performed during the 2-week, 3-month, and 2-year studies using HPLC, and no degradation of the bulk chemical was detected.

Methylcellulose

For the 2-week studies, methylcellulose was obtained from Spectrum Quality Products (Gardena, CA) in one lot (QG1176). The identity of lot QC1176 was confirmed using IR spectroscopy; the spectrum was consistent with the structure of methylcellulose. The average methoxyl content was 31.3%.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing ginseng with 0.5% methylcellulose (2-week studies) or sterile water (3-month and 2-year studies) to give the required concentrations. The vehicle for the 2-week study was selected as 0.5% methylcellulose with the anticipation that higher doses of ginseng might be needed in the 3-month and 2-year studies, which in methylcellulose might form suspensions rather than solutions. During the 2-week study, methylcellulose formulations demonstrated a tendency to form microbial growth after 2 weeks. Further work for the 3-month and 2-year studies showed that sterile water provided better control of microbial growth and accommodated higher formulation concentrations. The dose formulations for the 2-week studies were stored at approximately 5° C in amber glass bottles sealed with Teflon®-lined lids for up to 9 days. The dose formulations for the 3-month and 2 year studies were stored at less than or equal to –20° C in sealed Nalgene® plastic bottles for up to 39 or 44 days, respectively.

The analytical chemistry laboratory determined that a 400 mg/mL dose formulation in 0.5% methylcellulose was gavagable using 16- to 25-gauge needles.

Stability studies of a 2.5 mg/mL formulation in 0.5% methylcellulose or sterile water were conducted by the analytical chemistry laboratory and the study laboratory, respectively; all analyses used HPLC. Stability of dose formulation in 0.5% methylcellulose was confirmed for up to 42 days in sealed amber glass bottles at room temperature or 5° C, and for up to 3 hours under simulated animal room conditions. Stability of dose formulations in sterile water was confirmed for up to 45 days in sealed plastic bottles protected from light at room temperature, 5° C, and less than or equal to –20° C, but microbial growth occurred in samples stored at the two higher temperatures.

Periodic analyses of the dose formulations of ginseng were conducted by the study laboratory using HPLC; this system separated the seven ginsenosides; Rg1 was selected as the appropriate marker for formulation analysis. During the 2-week studies, the dose formulations were analyzed once; all six of the dose formulations analyzed were within 10% of the target concentrations. Animal room samples of these dose formulations were also analyzed; four of five rat animal room samples and all five mouse animal room samples were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed. Of the dose formulations analyzed, all 26 were within 10% of the target concentrations; all 16 rat and 15 mouse animal room samples were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 3 months. Of the dose formulations analyzed, 75 of 76 were within 10% of the target concentrations; all nine rat and all nine mouse animal room samples were within 10% of the target concentrations.

2-WEEK STUDIES

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 11 days and were 5 to 6 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. Groups of five male and five female rats and mice were administered ginseng by gavage in a 0.5% aqueous methylcellulose solution at doses of 0, 125, 250, 500, 1,000, or 2,000 mg/kg, 5 days a week for 16 (rats) or 17 (mice) days. Doses were administered at a volume of 5 (rats) or 10 (mice) mL/kg. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded daily for rats and mice. Animals were weighed on days 1 and 8 and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

At the end of the 2-week studies, necropsies were performed on all rats and mice. The adrenal glands, heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations of selected tissues were performed on vehicle control and 2,000 mg/kg rats and mice; these tissues were examined to a no-effect level in the remaining dosed groups. Table 2 lists the tissues and organs examined.

3-MONTH STUDIES

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

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 11 (male rats), 12 (female rats), 13 (female mice), or 14 (male mice) days and were approximately 5 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At 4 weeks and at the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were administered ginseng by gavage in sterile water at doses of 0, 1,000, 2,000, 3,000, 4,000, or 5,000 mg/kg, 5 days per week for 14 weeks. Groups of 10 male and 10 female clinical pathology rats received the same doses for 23 days. Dosing volumes were 5 (rats) or 10 (mice) mL/kg. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded weekly for core study rats and mice. Core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Animals were anesthetized with a carbon dioxide/ oxygen mixture, and blood was collected from the retroorbital sinus of clinical pathology rats on study days 4 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats) analyses. On the day prior to scheduled blood collection, rats were placed in individual polycarbonate cages; rats were bled approximately 2 hours after dosing on scheduled blood collection days. Blood collected for hematology determinations was placed in micro-collection tubes containing EDTA. The samples were gently inverted to prevent clotting prior to analysis. Blood collected for clinical chemistry was placed in serum separator tubes, centrifuged, and the serum was divided into two portions, one for corticosterone analyses and one for clinical chemistry. Hematology determinations were performed on an ADVIA 120 Hematology System (Bayer Diagnostics; Tarrytown, NY). All clinical chemistry analyses, except for corticosterone concentrations, were performed on a Hitachi 911 (Boehringer Mannheim; Indianapolis, IN). Serum corticosterone concentrations were determined using a double antibody, ^{125}iodine radioimmunoassay method using a Packard Cobra II Automatic Gamma counter (Packard Instrument Company; Downers Grove, IL) and reagents from ICN Biomedicals (Costa Mesa, CA). 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 and mice administered 0, 3,000, 4,000, or 5,000 mg/kg. The parameters evaluated are listed in Table 2. For 12 consecutive days prior to scheduled terminal sacrifice, 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 animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on vehicle control and 5,000 mg/kg rats and mice; the thymus was examined in all groups of male mice. Table 2 lists the tissues and organs routinely examined.

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

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were administered ginseng by gavage in sterile water at doses of 0, 1,250, 2,500, or 5,000 mg/kg, 5 days per week for 104 (male rats) or 105 (female rats and male and female mice) weeks; dosing volume was 5 (rats) or 10 (mice) mL/kg.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Animals were quarantined for 13 (male rats), 14 (female rats), 18 (female mice), or 19 (male 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 and mice were approximately 6 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats were housed three (male) or five (female) per cage, and mice were housed one (male) or three to 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; animals were weighed initially, weekly for the first 13 weeks, monthly thereafter, and at the end of the studies. Clinical findings were recorded at 4 or 5 weeks, monthly thereafter, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The reports, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the skin of male rats and mammary gland of female rats; no target organs were identified for mice.

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

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of Ginseng

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species		
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
11 days	Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days	Rats: 13 (males) or 14 (females) days Mice: 18 (females) or 19 (males) days
Average Age When Studies Began		
5 to 6 weeks	Rats: 5 to 6 weeks Mice: 6 to 7 weeks	6 to 7 weeks
Date of First Dose		
October 7, 2002	Rats: March 10 (males) or 11 (females), 2003 Mice: March 12 (females) or 13 (males), 2003	Rats: January 21 (males) or 22 (females), 2004 Mice: February 9 (females) or 10 (males), 2004
Duration of Dosing		
5 days per week for 16 (rats) or 17 (mice) days	5 days per week for 14 weeks	Rats: 5 days per week for 104 (males) or 105 (females) weeks Mice: 5 days per week for 105 weeks
Date of Last Dose		
October 22 (rats) or 23 (mice), 2002	Rats: June 9 (males) or 10 (females), 2003 Mice: June 11 (females) or 12 (males), 2003	Rats: January 17 (males) or 19 (females), 2006 Mice: February 7 (females) or 9 (males), 2006
Necropsy Dates		
October 23 (rats) or 24 (mice), 2002	Rats: June 10 (males) or 11 (females), 2003 Mice: June 12 (females) or 13 (males), 2003	Rats: January 16-18 (males) or 18-20 (females) 2006 Mice: February 8-10 (males) or 6-8 (females), 2006
Average Age at Necropsy		
8 to 9 weeks	19 to 20 weeks	Rats: 109 to 111 weeks Mice: 110 to 112 weeks
Size of Study Groups		
5 males and 5 females	10 males and 10 females	50 males and 50 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage		
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 3 (males) or 5 (females) Mice: 1 (males) or 3 to 5 (females)
Method of Animal Identification		
Tail tattoo	Tail tattoo	Tail tattoo

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of Ginseng

2-Week Studies	3-Month Studies	2-Year Studies
Diet Irradiated NTP-2000 open formula meal/pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed once a week	Same as 2-week studies	Same as 2-week studies
Water Tap water (City of Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Cages Polycarbonate cages (Lab Products, Inc., Seaford, DE), changed weekly (male mice) or twice weekly	Same as 2-week studies	Same as 2-week studies
Bedding Irradiated Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly (male mice) or twice weekly	Same as 2-week studies	Same as 2-week studies
Rack Filters Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks	Same as 2-week studies	Same as 2-week 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	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Doses 0, 125, 250, 500, 1,000, or 2,000 mg/kg in 0.5% methylcellulose by gavage (dosing volumes 5 mL/kg for rats and 10 mL/kg for mice)	0, 1,000, 2,000, 3,000, 4,000, or 5,000 mg/kg in sterile water by gavage (dosing volumes 5 mL/kg for rats and 10 mL/kg for mice)	0, 1,250, 2,500, or 5,000 mg/kg in sterile water by gavage (dosing volumes 5 mL/kg for rats and 10 mL/kg for mice)
Type and Frequency of Observation Observed twice daily; animals were weighed on days 1 and 8 and at the end of the studies; clinical findings were recorded daily.	Observed twice daily; core study animals weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly for core study animals.	Observed twice daily; animals were weighed initially, weekly for the first 13 weeks, monthly thereafter, and at the end of the studies; clinical findings were recorded at 4 or 5 weeks, monthly thereafter, and at the end of the studies.
Method of Sacrifice Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy Necropsies were performed on all animals. Organs weighed were adrenal glands, heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.

TABLE 2
Experimental Design and Materials and Methods in the Gavage Studies of Ginseng

2-Week Studies	3-Month Studies	2-Year Studies
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials. ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, bile acids, and corticosterone.	None
Histopathology		
Histopathology was performed on vehicle control and 2,000 mg/kg rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, brain, kidney, liver, and lung.	Complete histopathology was performed on vehicle control and 5,000 mg/kg 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, eye, gallbladder (mice only), Harderian gland, heart with aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology was performed on core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice only), Harderian gland, heart with aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, sperm samples were collected from male rats and mice in the vehicle control, 3,000, 4,000, and 5,000 mg/kg groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from vehicle control, 3,000, 4,000, and 5,000 mg/kg female rats and mice for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

STATISTICAL METHODS

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A3, B1, B3, C1, C3, D1, and D3 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

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

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

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis.

Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses. Proportions of regular cycling females in each exposed group were compared to the control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each exposure group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among exposure groups and between the control group and each exposed group was tested using chi-square statistics.

Historical Control Data

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

QUALITY ASSURANCE METHODS

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

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

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

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

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies (Witt *et al.*, 2000). Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS

2-WEEK STUDY

All rats survived to the end of the study (Table 3). Mean body weight gain of 2,000 mg/kg males was significantly greater than that of the vehicle controls. There were no clinical findings attributed to the administration of ginseng. No biologically significant organ weight changes occurred in dosed groups of rats (Table G1). There were no chemical-related gross or microscopic findings attributed to the administration of ginseng.

Dose Selection Rationale: Due to the lack of toxicity in the 2-week study, doses selected for the 3-month gavage study in rats were 1,000, 2,000, 3,000, 4,000, and 5,000 mg/kg.

TABLE 3
Survival and Body Weights of Rats in the 2-Week Gavage Study of Ginseng

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	99 ± 4	175 ± 5	76 ± 2	
125	5/5	100 ± 3	177 ± 4	78 ± 3	101
250	5/5	99 ± 3	178 ± 3	80 ± 2	102
500	5/5	100 ± 3	181 ± 4	82 ± 3	103
1,000	5/5	98 ± 4	175 ± 5	77 ± 2	100
2,000	5/5	98 ± 2	183 ± 3	85 ± 2*	105
Female					
0	5/5	90 ± 3	129 ± 3	39 ± 3	
125	5/5	92 ± 4	133 ± 3	41 ± 5	103
250	5/5	91 ± 4	136 ± 6	45 ± 4	105
500	5/5	91 ± 4	131 ± 2	40 ± 3	102
1,000	5/5	91 ± 4	130 ± 3	39 ± 2	101
2,000	5/5	89 ± 5	129 ± 3	40 ± 2	100

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

3-MONTH STUDY

All rats survived to the end of the study (Table 4). Final mean body weights and body weight gains of all dosed groups were similar to those of the vehicle control groups (Table 4 and Figure 2). Soft feces were evidence of occasional diarrhea in 4,000 and 5,000 mg/kg males and 3,000 mg/kg or greater females, but this diarrhea did not affect body weight, overall health, or behavior of the affected animals.

There were no changes in hematology, clinical chemistry (including serum corticosterone concentrations), or organ weights that were considered attributable to ginseng administration (Tables F1 and G2). There were no significant differences in sperm parameters of male rats or estrous cycles of female rats administered 3,000, 4,000, or 5,000 mg/kg when compared to the vehicle controls (Tables H1 and H2).

No lesions were observed by gross or histopathologic examinations that were attributed to the administration of ginseng.

Dose Selection Rationale: The highest dose of ginseng used in the 3-month study (5,000 mg/kg) was not toxic but was determined to be at the limit of gavagability. Accordingly, doses selected for the 2-year gavage study in rats were 1,250, 2,500, and 5,000 mg/kg.

TABLE 4
Survival and Body Weights of Rats in the 3-Month Gavage Study of Ginseng

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	107 ± 2	354 ± 6	248 ± 6	
1,000	10/10	107 ± 4	365 ± 8	258 ± 5	103
2,000	10/10	107 ± 3	351 ± 5	245 ± 5	99
3,000	10/10	107 ± 3	345 ± 5	238 ± 4	97
4,000	10/10	108 ± 3	354 ± 6	246 ± 7	100
5,000	10/10	107 ± 3	339 ± 8	233 ± 9	96
Female					
0	10/10	98 ± 2	206 ± 5	108 ± 4	
1,000	10/10	98 ± 2	202 ± 4	104 ± 4	98
2,000	10/10	98 ± 2	199 ± 3	100 ± 3	97
3,000	10/10	98 ± 2	205 ± 3	107 ± 2	100
4,000	10/10	99 ± 2	210 ± 5	111 ± 5	102
5,000	10/10	98 ± 2	201 ± 5	103 ± 4	98

[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.

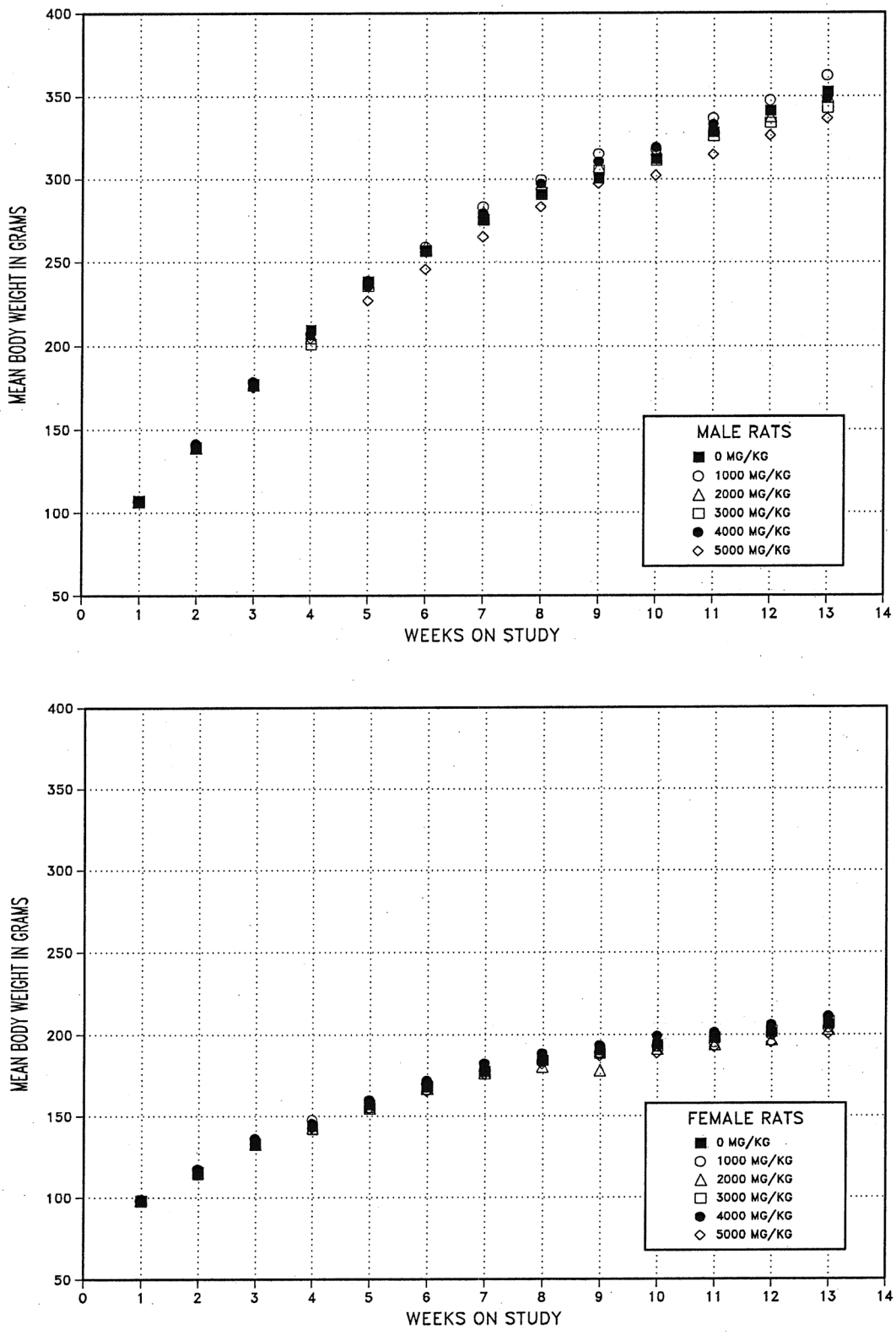


FIGURE 2
Growth Curves for Rats Administered Ginseng by Gavage for 3 Months

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 3). Nineteen dosed rats, five males and 14 females, had no gross or microscopic findings to explain the cause of death; the role of ginseng is unknown. Cause of death was recorded for all vehicle control males and females. Survival of female rats was reduced in the 1,250 mg/kg group and significantly reduced in the 5,000 mg/kg group. The reduced survival in females was associated with a variety of neoplasms and nonneoplastic lesions, none of which occurred in a chemical-related pattern.

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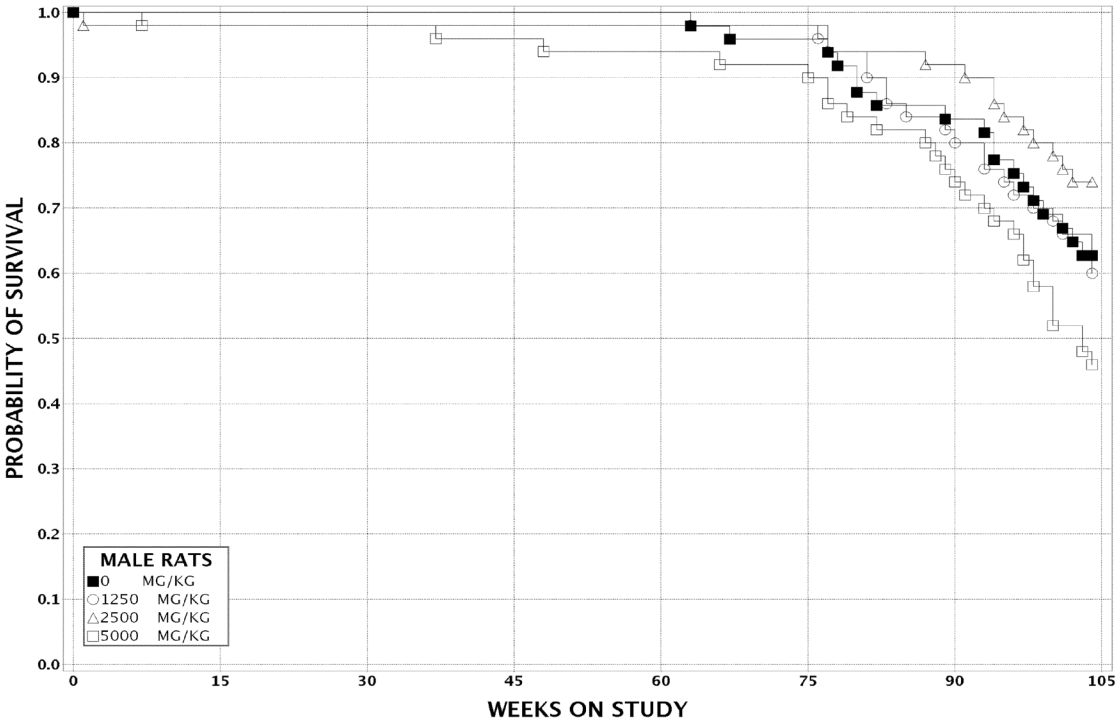
TABLE 5
Survival of Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	2	0	0	0
Moribund	13	16	7	16
Natural deaths	5	4	6	11
Animals surviving to study termination	30	30	37	23
Percent probability of survival at end of study[b]	63	60	74	46
Mean survival (days)[c]	679	686	693	651
Survival analysis[d]	P=0.108	P=0.934	P=0.299N	P=0.129
Female				
Animals initially in study	50	50	50	50
Accidental deaths	0	0	3	1
Moribund	8	12	7	10
Natural deaths	6	11	6	15
Animals surviving to study termination	36	27	34	24
Percent probability of survival at end of study	72	54	73	49
Mean survival (days)	700	646	662	655
Survival analysis	P=0.065	P=0.065	P=1.000	P=0.022

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



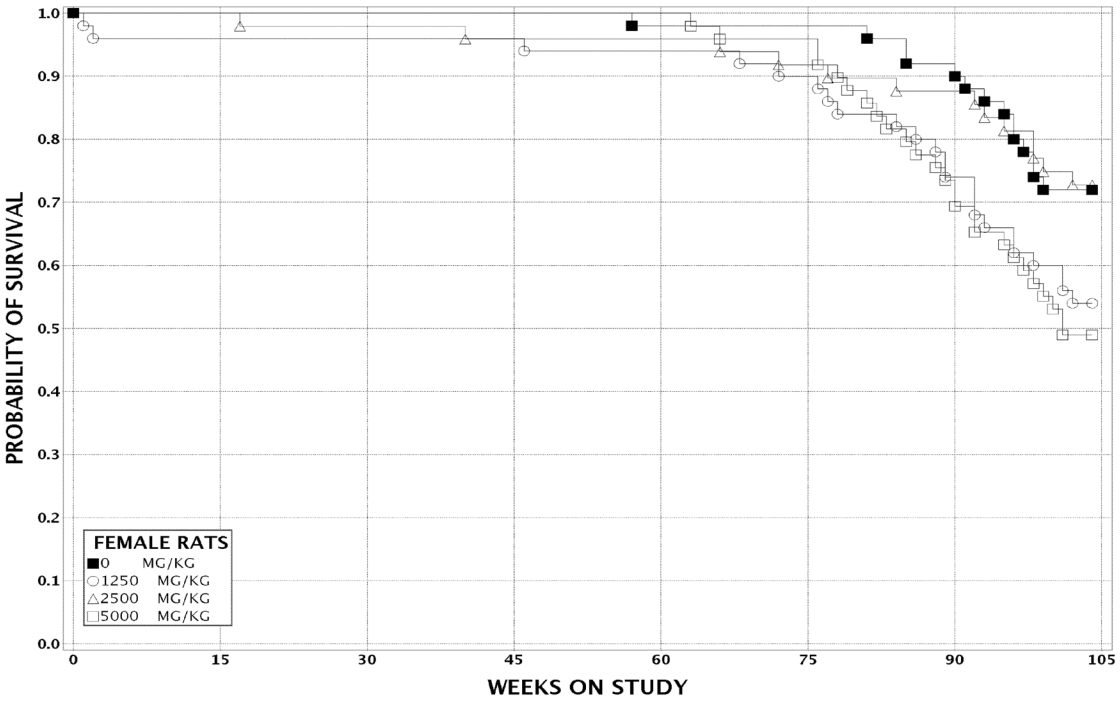
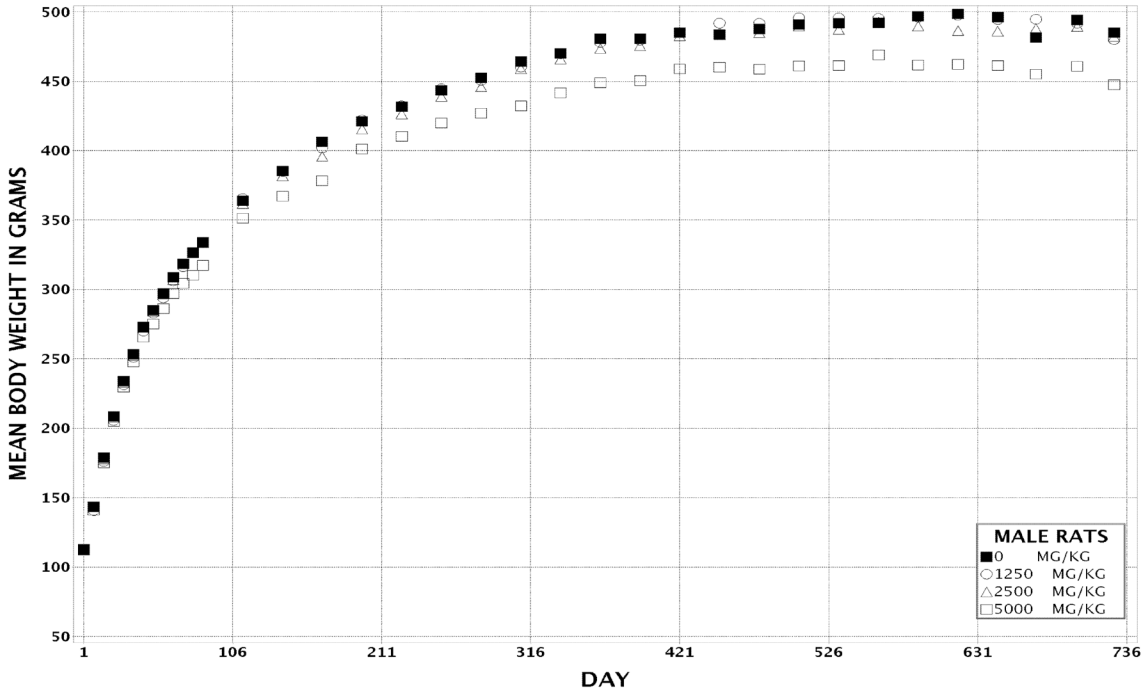


FIGURE 3
Kaplan-Meier Survival Curves for Rats
Administered Ginseng by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of 5,000 mg/kg females were less than those of the vehicle controls after week 61 of the study, and mean body weights of all other dosed groups of males and females were generally similar to those of the vehicle controls throughout the study (Figure 4; Tables 6 and 7). Intermittent diarrhea occurred in 5,000 mg/kg female rats during year 2 of the study, but this clinical finding did not affect the overall behavior or health of the animals.



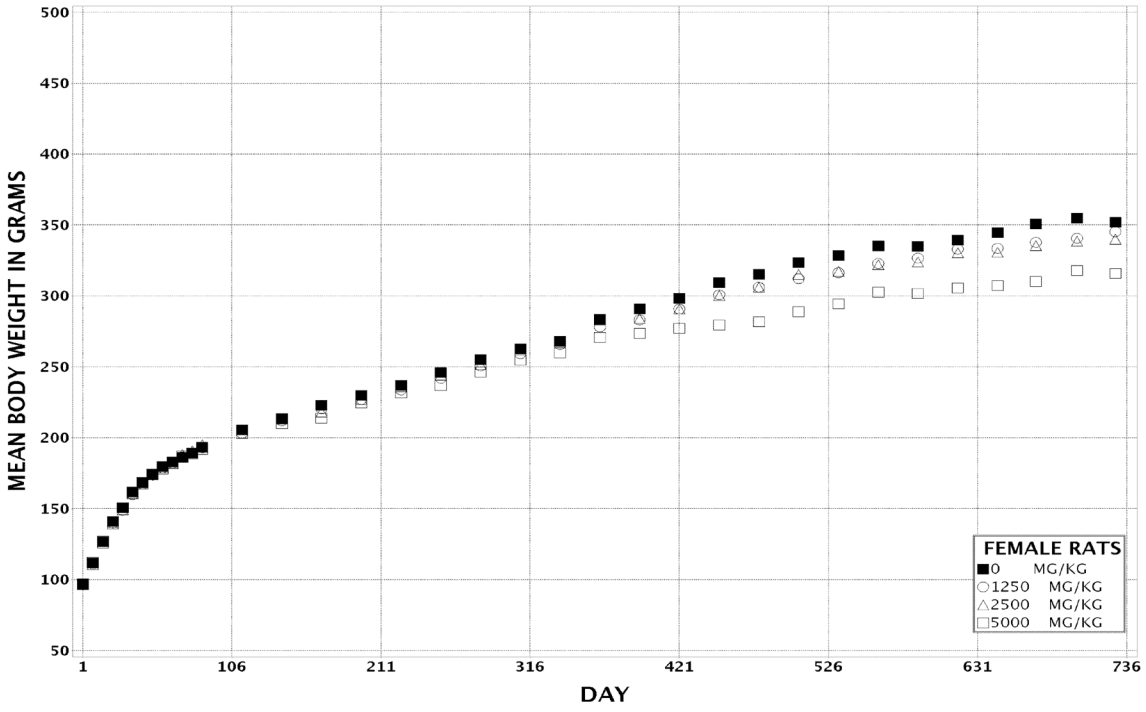


FIGURE 4
Growth Curves for Rats Administered Ginseng by Gavage for 2 Years

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of Ginseng

Days on Study	Vehicle Control		1,250 mg/kg			2,500 mg/kg			5,000 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	113	50	112	100	50	112	99	50	113	100	50
8	143	50	141	98	50	143	100	49	141	98	50
15	179	50	176	98	50	178	100	49	175	98	50
22	209	50	206	99	50	208	100	49	205	98	50
29	234	50	231	99	50	233	100	49	230	98	50
36	254	50	251	99	50	253	100	49	248	98	50
43	273	50	270	99	50	273	100	49	266	97	50
50	285	50	283	99	50	284	100	49	275	96	49
57	297	50	294	99	50	297	100	49	286	96	49
64	309	50	306	99	50	307	100	49	297	96	49
71	319	50	316	99	50	319	100	49	304	96	49
78	327	50	327	100	50	326	100	49	310	95	49
85	334	50	334	100	50	334	100	49	317	95	49
113	364	50	366	101	50	362	100	49	352	97	49
141	386	50	385	100	50	382	99	49	367	95	49
169	406	50	402	99	50	396	97	49	379	93	49
197	421	50	422	100	50	416	99	49	401	95	49
225	432	50	432	100	50	427	99	49	410	95	49
253	444	50	445	100	50	439	99	49	420	95	49
281	453	50	451	100	50	446	99	49	427	94	48
309	464	50	460	99	50	459	99	49	432	93	48
337	470	50	470	100	50	466	99	49	442	94	47
365	481	50	478	100	50	474	99	49	449	93	47
393	481	50	480	100	50	476	99	49	450	94	47
421	485	49	485	100	50	483	100	49	459	95	47
449	484	48	492	102	49	485	100	49	460	95	47
477	488	47	492	101	49	485	99	49	459	94	46
505	491	47	496	101	49	490	100	49	461	94	46
533	492	46	496	101	48	488	99	48	462	94	45
561	492	43	495	101	45	493	100	47	469	95	42
589	497	42	496	100	42	490	99	47	462	93	41
617	499	41	498	100	41	487	98	46	462	93	38
645	496	41	495	100	38	486	98	45	461	93	36
672	482	36	495	103	36	488	101	42	455	95	33
701	494	32	492	100	33	490	99	39	461	93	26
Mean for weeks											
1-13	252		250	99		251	100		244	97	
14-52	427		426	100		421	99		403	94	
53-101	489		491	100		486	99		459	94	

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of Ginseng

Days on Study	Vehicle Control		1,250 mg/kg			2,500 mg/kg			5,000 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	97	50	96	100	50	97	100	50	97	100	50
8	112	50	111	99	49	111	99	50	112	100	50
15	127	50	127	100	48	126	99	49	127	100	50
22	141	50	140	99	48	139	99	49	140	99	50
29	151	50	149	99	48	149	99	49	151	100	50
36	162	50	160	99	48	160	99	49	161	100	50
43	168	50	167	100	48	168	100	49	169	100	50
50	174	50	174	100	48	174	100	49	174	100	50
57	180	50	179	99	48	179	100	49	178	99	50
64	183	50	182	99	48	182	100	49	182	99	49
71	186	50	187	100	48	188	101	49	187	101	49
78	189	50	189	100	48	191	101	49	189	100	49
85	193	50	193	100	48	195	101	49	192	99	49
113	206	50	203	99	48	206	100	49	203	99	49
141	214	50	212	99	48	213	100	48	210	98	49
169	223	50	220	99	48	218	98	48	214	96	49
197	230	50	227	99	48	227	99	48	225	98	49
225	237	50	234	99	48	236	100	48	232	98	49
253	246	50	242	98	48	244	99	48	237	96	49
281	255	50	251	98	48	252	99	47	247	97	49
309	263	50	259	99	48	262	100	47	255	97	49
337	268	50	266	99	47	268	100	47	260	97	49
365	284	50	278	98	47	283	100	47	271	96	49
393	291	50	283	97	47	284	98	47	274	94	49
421	298	49	291	98	47	291	98	47	277	93	49
449	309	49	301	97	47	300	97	47	280	90	48
477	315	49	306	97	46	306	97	46	282	89	47
505	324	49	312	97	45	315	98	44	289	89	47
533	329	49	316	96	43	317	97	43	295	90	45
561	335	48	323	96	42	322	96	43	303	90	43
589	335	48	327	98	41	324	97	42	302	90	40
617	339	46	333	98	39	331	97	42	306	90	37
645	345	44	334	97	34	331	96	41	308	89	32
672	351	40	338	96	31	336	96	38	310	88	30
701	355	36	341	96	29	339	95	35	318	90	24
Mean for weeks											
1-13	159		158	99		158	99		158	99	
14-52	238		235	99		236	99		231	97	
53-101	324		314	97		314	97		293	90	

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 mammary gland and nose. 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.

Mammary Gland: The incidence of fibroadenoma (including multiple) in females occurred with a negative trend (P=0.001N) and was significantly decreased in the 5,000 mg/kg group (vehicle control, 32/50; 1,250 mg/kg, 30/50, P=0.518; 2,500 mg/kg, 30/50, P=0.589N; 5,000 mg/kg, 16/50, P=0.002N; Tables B1 and B2). The incidence of mammary gland fibroadenoma in the 5,000 mg/kg group was less than the historical control range for water gavage studies [94/150 (mean ± standard deviation, 63% ± 4%), range 58%-66%]; however, the incidence was within the historical range for all routes [701/1,350 (52% ± 15%), range 24%-86%]. Analysis by a model that adjusts for reduced group survival (Haseman *et al.*, 1997) indicated that the decreased incidence of mammary gland fibroadenoma observed in the 5,000 mg/kg group could only partly be attributed to decreased survival and may have been affected by ginseng exposure. Mammary gland fibroadenoma is a common finding in aged F344/N rats. Mammary gland fibroadenomas were not accompanied by increased incidences of adenoma or carcinoma. Mammary gland hyperplasia was seen in only two 1,250 mg/kg females (Table B3).

Fibroadenomas were benign neoplasms that consisted of proliferating neoplastic fibrous and glandular tissues. They were typically fairly large, well-demarcated, and expansive masses that retained the overall histological appearance of normal mammary glands with increased glandular tissue in well-defined lobules interspersed in various amounts of abundant fibrous connective tissues. The neoplastic epithelial cells were generally uniform in size and shape and formed clusters of acini lined by a single layer of neoplastic cells. These cells had round or oval nuclei, few mitotic figures, and minimal atypia. The cytoplasm was eosinophilic with vacuolation ranging from small fine vacuoles and a foamy appearance to coarse larger vacuoles. Often the acini became cystic as a result of being filled with eosinophilic fluid. Occasionally, there were ductular structures having single layers of columnar or cuboidal neoplastic cells.

Nose: In females, the incidences of minimal to mild inflammation of the respiratory epithelium were significantly increased in the 5,000 mg/kg group (vehicle control, 3/50; 1,250 mg/kg, 2/50; 2,500 mg/kg, 1/50; 5,000 mg/kg, 10/50; Table B3). Inflammation of the respiratory epithelium consisted of a variety of inflammatory responses and cell infiltrates including neutrophils, macrophages, and lymphocytes with occasional fibrosis.

MICE
2-WEEK STUDY

All mice survived to the end of the study (Table 8). The final mean body weight of 1,000 mg/kg males was significantly less than that of the vehicle controls. There were no clinical findings or organ weight differences attributed to the administration of ginseng. There were no significant chemical-related gross or histopathologic changes in dosed mice.

Dose Selection Rationale: Due to the lack of toxicity in the 2-week study, doses selected for the 3-month gavage study in mice were 1,000, 2,000, 3,000, 4,000, and 5,000 mg/kg.

TABLE 8
Survival and Body Weights of Mice in the 2-Week Gavage Study of Ginseng

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	22.9 ± 0.2	25.6 ± 0.3	2.8 ± 0.2	
125	5/5	22.6 ± 0.2	25.1 ± 0.2	2.5 ± 0.3	98
250	5/5	22.9 ± 0.2	25.6 ± 0.4	2.7 ± 0.3	100
500	5/5	23.0 ± 0.3	25.9 ± 0.4	2.9 ± 0.4	101
1,000	5/5	22.5 ± 0.2	24.4 ± 0.3*	1.9 ± 0.2	95
2,000	5/5	22.8 ± 0.3	24.8 ± 0.3	2.0 ± 0.1	97
Female					
0	5/5	18.1 ± 0.3	19.7 ± 0.4	1.7 ± 0.1	
125	5/5	17.8 ± 0.4	19.7 ± 0.2	1.8 ± 0.2	100
250	5/5	17.8 ± 0.3	19.7 ± 0.3	1.9 ± 0.3	100
500	5/5	17.7 ± 0.3	20.0 ± 0.2	2.3 ± 0.4	102
1,000	5/5	18.0 ± 0.4	19.7 ± 0.3	1.7 ± 0.3	100
2,000	5/5	18.0 ± 0.3	19.8 ± 0.2	1.8 ± 0.4	101

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

3-MONTH STUDY

All mice survived to the end of the study (Table 9). Final mean body weights and body weight gains of all dosed groups were similar to those of the vehicle control groups (Table 9 and Figure 5). There were no clinical findings attributed to the administration of ginseng.

There were no changes in hematology or organ weights that were considered attributable to ginseng administration (Tables F2 and G4). There were no significant differences in sperm parameters of male mice or the estrous cyclicity of female mice administered 3,000, 4,000, or 5,000 mg/kg when compared to the vehicle controls (Tables H3 and H4).

Although sporadic incidences of lesions were observed in the vehicle control and 5,000 mg/kg groups, there were no chemical-related gross or microscopic findings.

Dose Selection Rationale: The highest dose of ginseng used in the 3-month study (5,000 mg/kg) was not toxic but was determined in the concurrent rat study to be at the limit of gavagability. Accordingly, doses selected for the 2-year gavage study in mice were 1,250, 2,500, and 5,000 mg/kg.

TABLE 9
Survival and Body Weights of Mice in the 3-Month Gavage Study of Ginseng

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.9 ± 0.4	35.6 ± 0.8	11.7 ± 0.6	
1,000	10/10	23.6 ± 0.2	36.5 ± 0.8	13.0 ± 0.7	103
2,000	10/10	23.8 ± 0.4	36.0 ± 0.8	12.2 ± 0.7	101
3,000	10/10	23.8 ± 0.3	37.9 ± 1.4	14.1 ± 1.1	106
4,000	10/10	23.7 ± 0.3	36.1 ± 0.7	12.4 ± 0.6	101
5,000	10/10	23.9 ± 0.3	36.5 ± 0.9	12.6 ± 0.7	103
Female					
0	10/10	18.9 ± 0.3	29.6 ± 1.0	10.7 ± 0.9	
1,000	10/10	19.5 ± 0.3	31.7 ± 1.0	12.2 ± 0.9	107
2,000	10/10	19.0 ± 0.3	30.3 ± 0.8	11.3 ± 0.7	102
3,000	10/10	19.0 ± 0.3	31.0 ± 0.8	12.0 ± 0.7	105
4,000	10/10	19.2 ± 0.3	28.1 ± 0.9	8.9 ± 0.8	95
5,000	10/10	18.8 ± 0.2	28.5 ± 0.7	9.8 ± 0.5	96

[a] Number of animals surviving at 3 months/number initially in group

[b] Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.

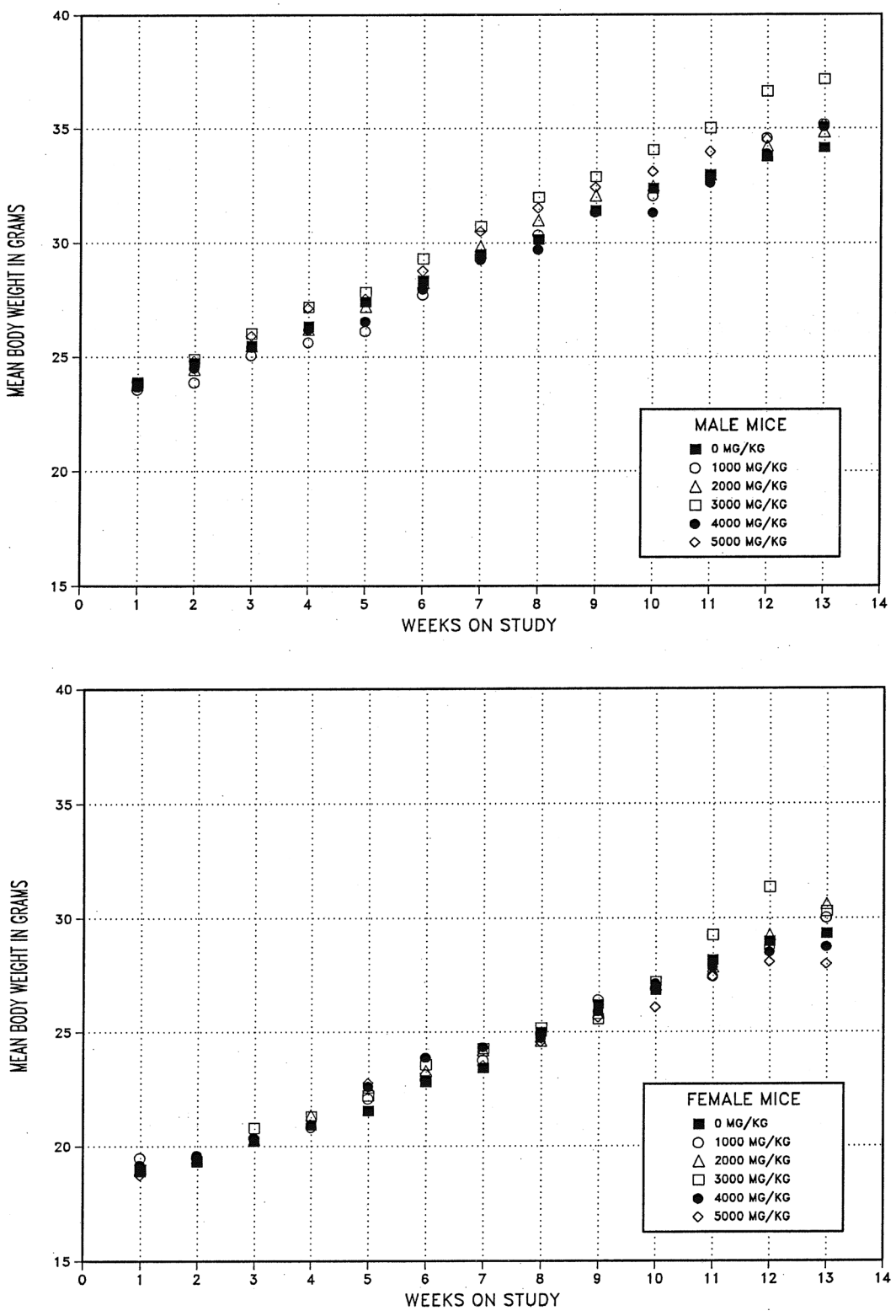


FIGURE 5
Growth Curves for Mice Administered Ginseng by Gavage for 3 Months

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 10 and in the Kaplan-Meier survival curves (Figure 6). Survival of dosed groups was similar to that of the vehicle control groups.

TABLE 10
Survival of Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	0	0	1
Moribund	12	10	8	8
Natural deaths	6	7	4	8
Animals surviving to study termination	32	33[d]	38	33[d]
Percent probability of survival at end of study[b]	64	64	76	66
Mean survival (days)[c]	691	693	707	673
Survival analysis[e]	P=0.892N	P=1.000	P=0.267	P=1.000
Female				
Animals initially in study	50	50	50	50
Accidental deaths	0	0	1	4
Moribund	7	11	10	8
Natural deaths	5	8	5	6
Animals surviving to study termination	38	31	34	32
Percent probability of survival at end of study	76	62	69	70
Mean survival (days)	711	689	667	657
Survival analysis	P=0.764	P=0.160	P=0.555	P=0.542

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

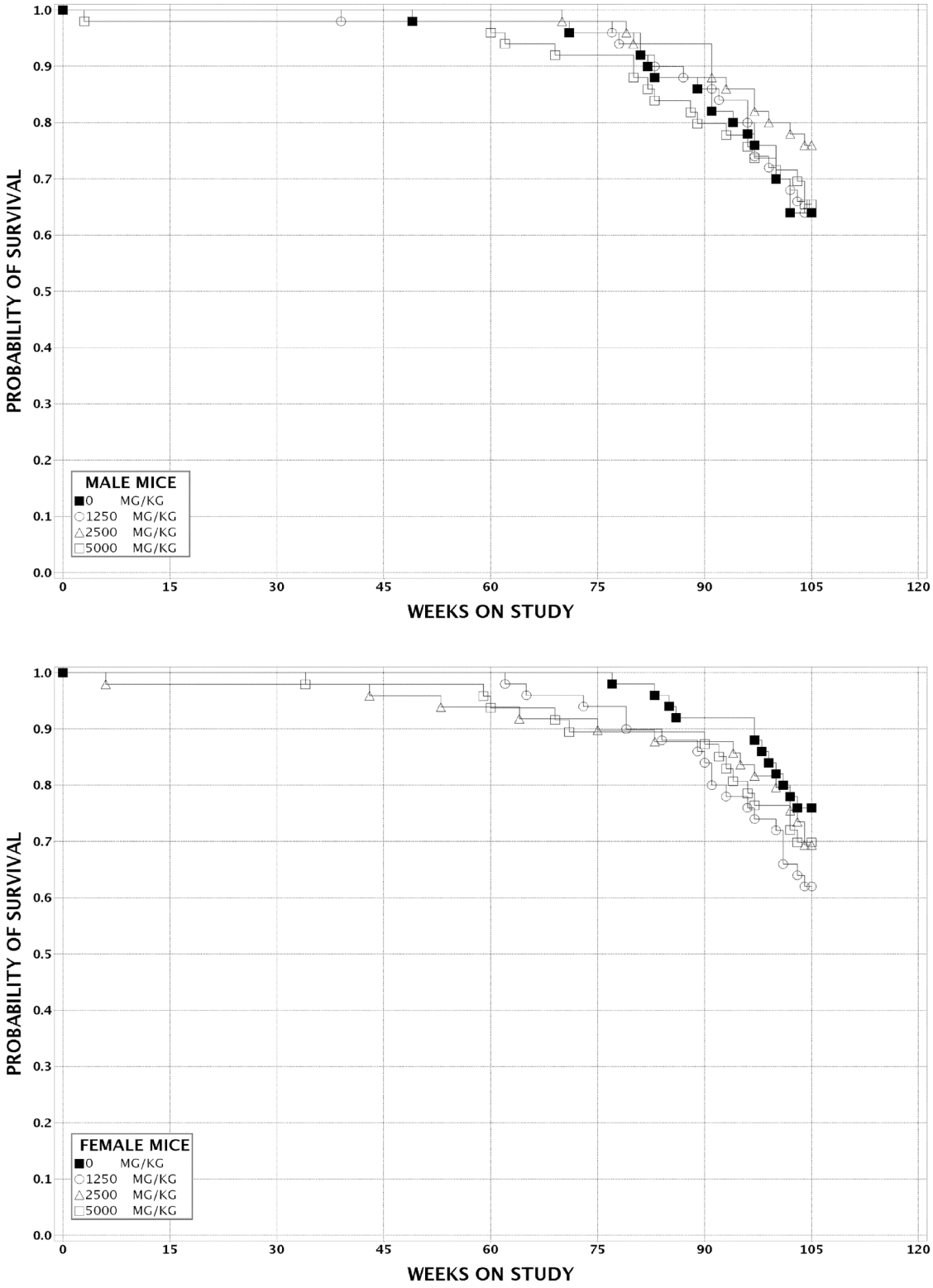
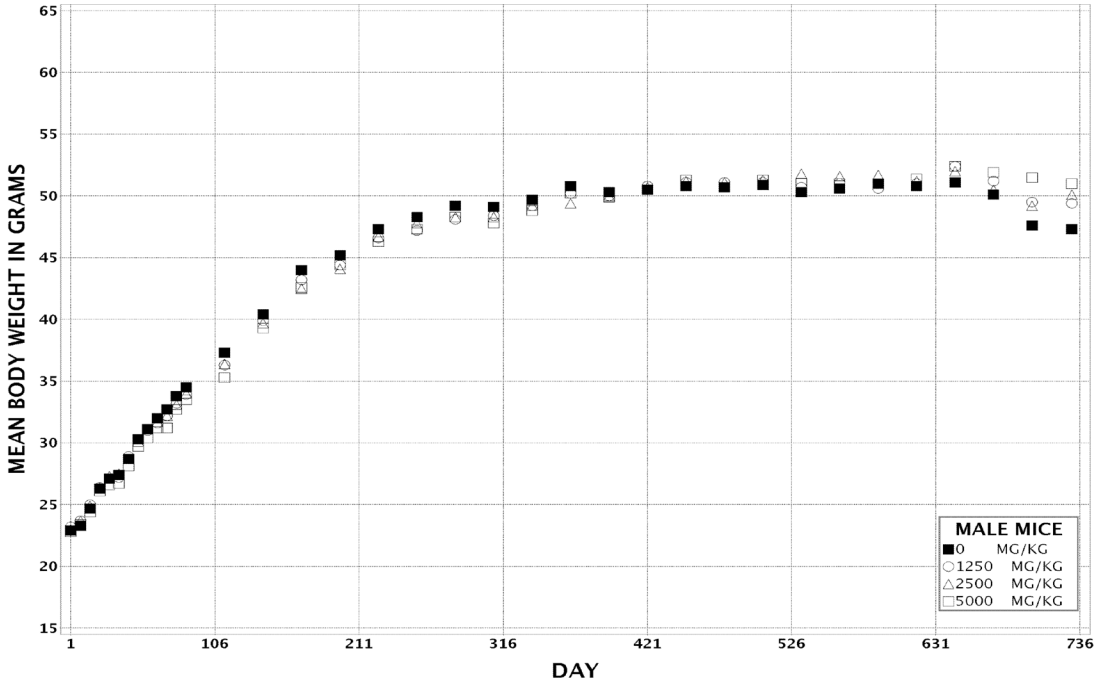


FIGURE 6
Kaplan-Meier Survival Curves for Mice Administered Ginseng by Gavage for 2 Years

Body Weights and Clinical Findings

Mean body weights of dosed males and females were similar to those of the vehicle controls during the 2-year study (Figure 7; Tables 11 and 12). Intermittent diarrhea occurred in 5,000 mg/kg males during the last 6 months of the study, but this ginseng-related clinical finding did not affect the body weight, overall behavior, or health of the animals.



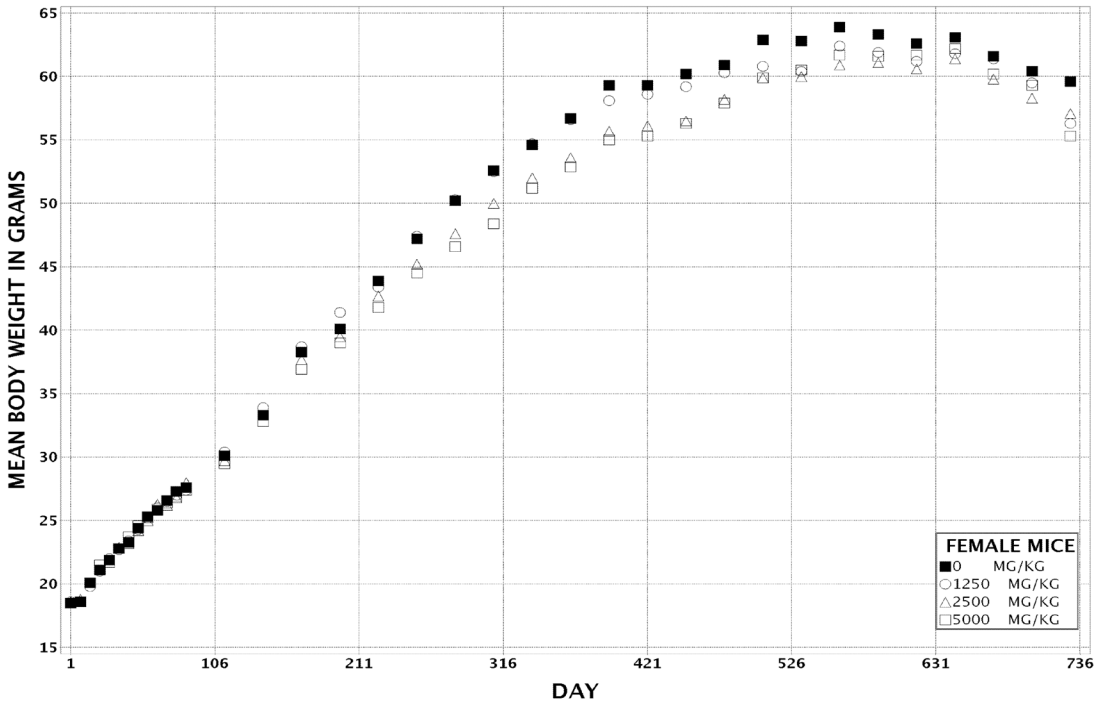


FIGURE 7
Growth Curves for Mice Administered Ginseng by Gavage for 2 Years

TABLE 11
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of Ginseng

Days on Study	Vehicle Control		1,250 mg/kg			2,500 mg/kg			5,000 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	22.9	50	23.2	102	50	23.0	101	50	22.8	100	50
8	23.3	50	23.7	102	50	23.7	102	50	23.4	100	50
15	24.7	50	25.0	101	50	24.9	101	50	24.4	99	50
22	26.3	50	26.4	100	50	26.4	100	50	26.1	100	49
29	27.1	50	27.1	100	50	27.3	101	50	26.6	98	49
36	27.4	50	27.2	99	50	27.5	100	50	26.7	97	49
43	28.7	50	28.9	100	50	28.7	100	50	28.1	98	49
50	30.3	50	30.3	100	50	30.1	100	50	29.7	98	49
57	31.1	50	31.0	100	50	31.1	100	50	30.4	98	49
64	32.0	50	31.6	99	50	31.7	99	50	31.2	98	49
71	32.7	50	32.2	99	50	32.2	99	50	31.2	96	49
78	33.8	50	33.2	98	50	33.1	98	50	32.7	97	49
85	34.5	50	33.9	98	50	34.0	98	50	33.5	97	49
113	37.3	50	36.3	97	50	36.4	98	50	35.3	95	49
141	40.4	50	39.9	99	50	39.7	98	50	39.3	97	49
169	44.0	50	43.2	98	50	42.5	97	50	42.6	97	49
197	45.2	50	44.4	98	50	44.1	98	50	44.4	98	49
225	47.3	50	46.6	99	50	46.7	99	50	46.3	98	49
253	48.3	50	47.2	98	50	47.8	99	50	47.3	98	49
281	49.2	50	48.1	98	49	48.3	98	50	48.3	98	49
309	49.1	50	48.4	99	49	48.3	98	50	47.8	97	49
337	49.7	50	49.2	99	49	49.2	99	50	48.8	98	49
365	50.8	49	50.2	99	49	49.4	97	50	50.2	99	49
393	50.3	49	50.0	99	49	50.0	100	50	49.9	99	49
421	50.5	49	50.8	101	49	50.7	100	50	50.5	100	48
449	50.8	49	51.1	101	49	51.2	101	50	51.3	101	47
477	50.7	49	51.1	101	49	51.1	101	50	50.7	100	47
505	50.9	48	51.1	100	49	51.3	101	49	51.3	101	46
533	50.3	48	50.7	101	49	51.8	103	49	51.0	101	46
561	50.6	48	51.0	101	47	51.6	102	47	51.0	101	44
589	51.0	44	50.6	99	45	51.7	102	47	51.0	100	41
617	50.8	43	51.0	101	44	51.2	101	47	51.4	101	39
645	51.1	41	52.4	103	42	52.0	102	44	52.4	103	39
673	50.1	38	51.2	102	40	50.5	101	43	51.9	103	37
701	47.6	35	49.5	104	35	49.2	103	40	51.5	108	35
Mean for weeks											
1-13	28.8		28.7	100		28.7	100		28.2	98	
14-52	45.6		44.8	98		44.8	98		44.5	98	
53-101	50.4		50.8	101		50.9	101		51.1	101	

TABLE 12
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of Ginseng

Days on Study	Vehicle Control		1,250 mg/kg			2,500 mg/kg			5,000 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	18.5	50	18.6	100	50	18.6	101	50	18.5	100	50
8	18.6	50	18.6	100	50	18.8	101	50	18.6	100	50
15	20.1	50	19.8	99	50	20.1	100	50	20.1	100	50
22	21.1	50	21.0	100	50	21.1	100	50	21.5	102	50
29	21.9	50	22.0	100	50	21.9	100	50	21.7	99	50
36	22.8	50	22.7	100	50	22.9	100	49	22.8	100	50
43	23.3	50	23.4	101	50	23.2	100	48	23.7	102	50
50	24.4	50	24.3	100	50	24.2	99	48	24.6	101	50
57	25.3	50	25.2	100	50	25.0	99	48	25.2	100	50
64	25.8	50	26.0	101	50	26.3	102	48	25.9	101	50
71	26.6	50	26.5	100	50	26.2	99	48	26.4	99	50
78	27.3	50	27.0	99	50	26.8	98	48	26.9	99	50
85	27.6	50	27.4	99	50	28.0	101	48	27.4	99	50
113	30.1	50	30.4	101	50	29.7	99	48	29.5	98	50
141	33.3	50	33.9	102	50	33.3	100	48	32.8	98	50
169	38.3	50	38.7	101	50	37.7	99	48	36.9	96	50
197	40.1	50	41.4	103	50	39.5	98	48	39.0	97	50
225	43.9	50	43.4	99	50	42.7	97	48	41.8	95	48
253	47.2	50	47.4	100	50	45.2	96	48	44.5	94	47
281	50.2	50	50.3	100	50	47.6	95	48	46.6	93	47
309	52.6	50	52.5	100	50	50.0	95	47	48.4	92	47
337	54.6	50	54.7	100	50	52.0	95	47	51.2	94	47
365	56.7	50	56.6	100	50	53.6	95	47	52.9	93	47
393	59.3	50	58.1	98	50	55.7	94	46	55.0	93	47
421	59.3	50	58.6	99	50	56.1	95	46	55.3	93	45
449	60.2	50	59.2	98	48	56.5	94	45	56.3	94	44
477	60.9	50	60.3	99	48	58.2	96	45	57.9	95	43
505	62.9	50	60.8	97	48	59.9	95	45	59.9	95	42
533	62.8	50	60.4	96	47	60.0	96	44	60.5	96	42
561	63.9	49	62.4	98	45	60.9	95	44	61.7	97	41
589	63.3	48	61.9	98	44	61.1	97	43	61.6	97	41
617	62.6	46	61.2	98	44	60.6	97	43	61.7	99	41
645	63.1	46	61.8	98	40	61.4	97	43	62.2	99	39
673	61.6	45	61.4	100	37	59.8	97	40	60.2	98	36
701	60.4	41	59.5	98	35	58.3	97	39	59.3	98	35
Mean for weeks											
1-13	23.3		23.3	100		23.3	100		23.3	100	
14-52	43.4		43.6	100		42.0	97		41.2	95	
53-101	61.3		60.2	98		58.6	96		58.8	96	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms of the lung and ovary. 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.

Lung: Incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) occurred with positive trends in males (P=0.008 and P=0.014; Table C2). Increased incidences in the 5,000 mg/kg group were not significant [adenoma: vehicle control, 9/50; 1,250 mg/kg, 3/50, P=0.056N; 2,500 mg/kg, 6/50, P=0.246N; 5,000 mg/kg, 16/50, P=0.080; adenoma or carcinoma (combined): 12/50; 6/50, P=0.091N; 10/50, P=0.362N; 19/50, P=0.089; Tables C1 and C2].

The incidence of alveolar/bronchiolar adenoma in the 5,000 mg/kg males was greater than the historical control range for water gavage studies [29/150, mean ± standard deviation (19% ± 1%), range 18%-20%] or all routes [228/1,449 (16% ± 6%), range 2%-30%]. The incidence of alveolar/ bronchiolar adenoma or carcinoma (combined) in 5,000 mg/kg males exceeded the historical control range for water gavage studies [44/150 (29% ± 5%), range 24%-32%], but was within the historical range for all routes [376/1,449 (26% ± 7%), range 14%-40%]. The increased incidence of alveolar/ bronchiolar adenoma in the 5,000 mg/kg group was not associated with alveolar or bronchiolar epithelial hyperplasia and did not appear to be related to the administration of ginseng.

Alveolar/bronchiolar adenomas were discrete hypercellular masses with well-demarcated borders having alveolar or papillary structures composed of well-differentiated large, cuboidal to round epithelial cells often forming papillary projections into the alveolar or bronchiolar lumens with distortion of the normal alveolar architecture and slight compression of the surrounding parenchyma. Alveolar/bronchiolar carcinomas were usually larger hypercellular masses with irregular borders and areas of solid cell growth or papillary structures having multiple cell layers that distorted the normal alveolar architecture. Carcinomas consisted of fairly pleomorphic, polygonal to columnar, sometimes atypical, epithelial cells with increased mitotic activity and variable compression or invasion of the surrounding parenchyma.

Ovary: Incidences of cystadenoma occurred with a negative trend (P=0.010N; Table D2) in female mice, and the incidence in the 5,000 mg/kg group was significantly decreased [vehicle control, 7/50; 1,250 mg/kg, 2/50, P=0.098N; 2,500 mg/kg, 3/50, P=0.193N; 5,000 mg/kg, 0/50, P=0.013N; Tables D1 and D2]. The incidences of cystadenoma in all groups were within the historical control ranges for water gavage studies and all study routes. However, the incidence in vehicle controls was at the high end of the historical control ranges for both water gavage studies [11/150 (7% ± 6%), range 2%-14%] and all routes [70/1,468 (5% ± 3%), range 0%-14%]. Therefore, these decreases do not appear related to the administration of ginseng.

Cystadenomas characteristically have fronds and branching papillae that extend into the lumen of cystic cavities. These papillae consist of thin fibrovascular tissue stalks that are lined by single or multiple layers of cuboidal or columnar epithelial cells. Single or multiple layers of epithelium usually line the wall of each cyst, and the lumen of the cyst often contains proteinaceous secretory material. The epithelial cells are non-ciliated, relatively uniform with little or no pleomorphism, and have basally located round to oval nuclei and moderate amounts of cytoplasm.

GENETIC TOXICOLOGY

Ginseng (with doses up to 3,333 µg/plate in the first study and 10,000 µg/plate in the second study) was not mutagenic in either of two independent bacterial mutagenicity assays, with or without exogenous metabolic activation (Table E1; Zeiger *et al*., 1992). Bacterial strains tested in the first study included *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, and TA1535, with and without 10% or 30% hamster or rat liver S9. Strains tested in the second study included *S. typhimurium* strains TA98 and TA100 and *Escherichia coli* strain WP2 *uvrA/*pKM101, with and without 10% rat liver S9. A precipitate was noted in the first study at doses of 1,000 and 3,333 µg/plate; however, this did not interfere with colony growth or enumeration. In addition to the negative results in the two bacterial tests, no significant increases in the frequencies of micronucleated normochromatic erythrocytes were seen in the peripheral blood of male or female B6C3F1 mice administered 1,000 to 5,000 mg/kg ginseng for 3 months by gavage (Table E2). The percentage of polychromatic erythrocytes in the peripheral blood of male and female mice was unaltered by chemical exposure, suggesting a lack of ginseng-associated bone marrow toxicity.

DISCUSSION AND CONCLUSIONS

The ginseng used in the present studies was a water/alcohol extract from the plant *Panax ginseng* C.A. Meyer in dried powder form. In the 2-week studies, rats and mice were administered 0, 125, 250, 500, 1,000, or 2,000 mg ginseng/kg body weight by gavage. There were no changes in body weights, survival, or histopathology between the dosed groups and the vehicle control groups in rats or mice. Since there were no effects at the high dose of 2,000 mg/kg in the 2-week studies, the highest dose was increased to 5,000 mg/kg, the highest permissible dose for gavage studies, for the 3-month studies in rats and mice. In the 3-month studies, all rats and mice survived, and there were no ginseng-related effects on body weights, organ weights, or histopathology. Rats and mice in the 2-year studies were administered 0, 1,250, 2,500, or 5,000 mg/kg by gavage. In the 2-year studies, other than in the 5,000 mg/kg female rats, mean body weights of dosed male and female rats and mice were similar to those of the vehicle controls. Mean body weights of 5,000 mg/kg female rats were 10% to 12% less than the vehicle controls during the last 6 months of the study. Survival of female rats was reduced in the 1,250 mg/kg group and significantly reduced in the 5,000 mg/kg group. The reduced survival in these female rats was associated with unknown effects unrelated to ginseng administration. The survival of the other dosed groups of male and female rats and mice was similar to that of the vehicle controls.

An oral LD_{50} of 750 mg ginseng/kg body weight for rats and 200 mg/kg for mice was reported in the literature (RTECS, 1998). The present studies showed that the oral LD_{50} for rats and mice exceeded 5,000 mg/kg. The difference may be due to the use of different ginseng preparations or the strains of test animals used.

In the 2-year studies, incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in male mice increased with a positive trend, but the incidence in the 5,000 mg/kg group was not significantly increased. The incidence was only slightly above the historical range for vehicle controls in water gavage studies (24%-32%) and was well within the historical control range for all routes (14%-40%). Therefore, the increase was not considered treatment related. No other increases in the incidences of neoplasms were observed in dosed rats or mice. Negative trends in the incidences of mammary gland fibroadenoma in female rats and ovarian cystadenoma in female mice occurred. The incidence of mammary gland fibroadenoma in 5,000 mg/kg females was significantly decreased; this decrease could only partly be attributed to the decreased survival in 5,000 mg/kg females.

Ginseng was not genotoxic in *Salmonella typhimurium* or *Escherichia coli* with or without S9 and did not induce increases in micronuclei in peripheral blood erythrocytes of male or female B6C3F1 mice administered ginseng by gavage for 3 months.

The results of these 2-year studies in rats and mice showed there were no toxic or carcinogenic effects. Epidemiologic studies have shown reduction in the risk of cancer development among people who regularly consumed ginseng (Yun and Choi, 1998); the decrease was inversely related to dose. The authors concluded that *Panax ginseng* C.A. Meyer has a preventive effect against cancer that is not organ specific. Experimentally, red ginseng extract has been reported to have an anticarcinogenic effect against pulmonary, liver, mammary gland, ovarian, and uterine cervix tumors induced by chemical carcinogens in mice (Yun *et al*., 1983; Bespalov *et al*., 1993, 2001; Yun and Choi, 1995, 1998, Shin *et al*., 2000; Yun, 2003, Panwar *et al*., 2005b). In the present studies, a dose concentration of 5,000 mg/kg did not induce neoplasms. On the other hand, the incidences of spontaneous neoplasms like mammary gland fibroadenoma in female rats and ovarian cystadenoma in female mice were decreased, although the relationship to ginseng administration was not clear. These findings do not necessarily support the anticarcinogenic activity of ginseng, especially since incidences of other spontaneous neoplasms commonly found in Fischer rats (i.e., mononuclear cell leukemia, pituitary gland pars distalis adenoma and carcinoma, testicular adenoma, thyroid gland C-cell adenoma, and uterine polyp) were not affected. In B6C3F1 mice, incidences of spontaneous Harderian gland, liver, and lung lesions were also not affected.

Overexposure to ginseng in humans has been named ginseng abuse syndrome (Siegel, 1979). The symptoms include hypertension, gastrointestinal disturbances, insomnia, nervousness, confusion, and depression (Kitts and Hu, 2000).

It has been postulated that the diverse effects of ginseng may be related to its modulation of hormones. The

present studies found no evidence of hormonal effects in rats and mice including effects on estrous cycles.

Based on the sperm motility and vaginal cytology evaluation results, the reproductive organ weights, and the histopathology of the reproductive organs of the 3-month study animals, there was no evidence of ginseng toxicity to the reproductive system of either rats or mice.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of ginseng in male or female F344/N rats or B6C3F1 mice administered 1,250, 2,500, or 5,000 mg/kg.

The incidence of mammary gland fibroadenoma was significantly decreased in 5,000 mg/kg female rats.

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

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

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

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	2			
Moribund	13	16	7	16
Natural deaths	5	4	6	11
Survivors				
Terminal sacrifice	30	30	37	23
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Adenocarcinoma			1 (2%)	
Adenoma		1 (2%)		
Liposarcoma		1 (2%)		
Intestine large, rectum	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma, multiple			1 (2%)	
Hepatocellular adenoma		1 (2%)		2 (4%)
Hepatocellular adenoma, multiple			1 (2%)	
Hepatocellular carcinoma	1 (2%)			
Hepatocellular carcinoma, multiple			1 (2%)	
Mesentery	(13)	(9)	(6)	(5)
Carcinoma, metastatic, kidney	1 (8%)			
Schwannoma malignant			1 (17%)	
Oral mucosa	(0)	(1)	(1)	(1)
Squamous cell carcinoma		1 (100%)		
Squamous cell papilloma			1 (100%)	1 (100%)
Pancreas	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney	1 (2%)			
Schwannoma malignant, metastatic, mesentery			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Sarcoma				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(0)	(1)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		2 (4%)		1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	3 (6%)	6 (12%)	6 (12%)	2 (4%)
Pheochromocytoma benign, multiple	1 (2%)	2 (4%)		1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	2 (4%)	
Parathyroid gland	(50)	(49)	(49)	(46)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Endocrine System (continued)								
Pituitary gland	(50)		(50)		(50)		(50)	
Pars distalis, adenoma	20	(40%)	15	(30%)	16	(32%)	16	(32%)
Thyroid gland	(50)		(50)		(50)		(50)	
Bilateral, C-cell, adenoma					1	(2%)		
C-cell, adenoma	11	(22%)	8	(16%)	5	(10%)	5	(10%)
C-cell, carcinoma					1	(2%)		
Follicular cell, adenoma			2	(4%)			1	(2%)
Follicular cell, carcinoma	1	(2%)						
General Body System								
None								
Genital System								
Epididymis	(50)		(50)		(50)		(50)	
Carcinoma, metastatic, kidney	1	(2%)						
Schwannoma malignant, metastatic, mesentery					1	(2%)		
Preputial gland	(50)		(50)		(50)		(50)	
Adenoma			1	(2%)				
Prostate	(50)		(50)		(50)		(50)	
Adenoma	1	(2%)						
Seminal vesicle	(50)		(50)		(50)		(50)	
Adenoma	1	(2%)						
Testes	(50)		(50)		(50)		(50)	
Interstitial cell, adenoma	13	(26%)	6	(12%)	10	(20%)	10	(20%)
Interstitial cell, adenoma, multiple	32	(64%)	38	(76%)	36	(72%)	36	(72%)
Hematopoietic System								
Bone marrow	(50)		(50)		(50)		(50)	
Lymph node	(7)		(15)		(4)		(6)	
Lymph node, mesenteric	(49)		(50)		(50)		(50)	
Schwannoma malignant, metastatic, mesentery					1	(2%)		
Spleen	(50)		(50)		(50)		(50)	
Thymus	(50)		(47)		(49)		(50)	
Integumentary System								
Mammary gland	(50)		(50)		(50)		(50)	
Fibroadenoma	2	(4%)	2	(4%)	2	(4%)		
Skin	(50)		(50)		(50)		(50)	
Epidermis, basal cell adenoma	1	(2%)	1	(2%)	2	(4%)	1	(2%)
Epidermis, basal cell carcinoma							1	(2%)
Epidermis, squamous cell carcinoma	1	(2%)						
Epidermis, squamous cell papilloma			1	(2%)	1	(2%)		
Pinna, neural crest tumor			1	(2%)			1	(2%)
Sebaceous gland, adenoma			1	(2%)				
Subcutaneous tissue, fibroma	2	(4%)	6	(12%)	2	(4%)	7	(14%)
Subcutaneous tissue, fibrosarcoma					1	(2%)	1	(2%)
Subcutaneous tissue, fibrous histiocytoma	1	(2%)						
Subcutaneous tissue, sarcoma					1	(2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Musculoskeletal System								
Bone	(50)		(50)		(50)		(50)	
Cranium, osteosarcoma			1	(2%)				
Femur, osteosarcoma	1	(2%)					1	(2%)
Mandible, squamous cell carcinoma, metastatic, oral mucosa			1	(2%)				
Rib, osteosarcoma					1	(2%)		
Skeletal muscle	(0)		(0)		(0)		(1)	
Alveolar/bronchiolar carcinoma, metastatic, lung							1	(100%)
Nervous System								
Brain	(50)		(50)		(50)		(50)	
Glioma benign					1	(2%)		
Granular cell tumor benign							1	(2%)
Peripheral nerve	(0)		(0)		(2)		(0)	
Respiratory System								
Larynx	(1)		(0)		(0)		(0)	
Lung	(50)		(50)		(50)		(50)	
Alveolar/bronchiolar adenoma					1	(2%)		
Alveolar/bronchiolar carcinoma							1	(2%)
Carcinoma, metastatic, Harderian gland	1	(2%)						
Carcinoma, metastatic, kidney	1	(2%)						
Carcinoma, metastatic, thyroid gland	1	(2%)						
Carcinoma, metastatic, Zymbal's gland							1	(2%)
Nose	(50)		(50)		(50)		(50)	
Adenoma							1	(2%)
Trachea	(50)		(50)		(50)		(50)	
Special Senses System								
Eye	(50)		(50)		(50)		(50)	
Harderian gland	(50)		(50)		(50)		(50)	
Carcinoma	1	(2%)						
Zymbal's gland	(0)		(0)		(0)		(1)	
Carcinoma							1	(100%)
Urinary System								
Kidney	(50)		(50)		(50)		(50)	
Renal tubule, adenoma	1	(2%)						
Renal tubule, carcinoma	1	(2%)						
Urinary bladder	(50)		(50)		(50)		(50)	
Systemic Lesions								
Multiple organs[b]	(50)		(50)		(50)		(50)	
Histiocytic sarcoma					2	(4%)		
Leukemia mononuclear	18	(36%)	25	(50%)	15	(30%)	12	(24%)
Mesothelioma malignant	2	(4%)	3	(6%)	4	(8%)	2	(4%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	50	49	48
Total primary neoplasms	116	126	117	106
Total animals with benign neoplasms	49	49	49	47
Total benign neoplasms	88	94	88	85
Total animals with malignant neoplasms	26	28	26	18
Total malignant neoplasms	28	31	29	20
Total animals with metastatic neoplasms	3	1	1	2
Total metastatic neoplasms	6	1	3	3
Total animals with uncertain neoplasms-benign or malignant		1		1
Total uncertain neoplasms		1		1

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

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	4/50 (8%)	8/50 (16%)	6/50 (12%)	3/50 (6%)
Adjusted rate[b]	9.4%	18.4%	13.1%	7.5%
Terminal rate[c]	2/30 (7%)	6/30 (20%)	5/37 (14%)	1/23 (4%)
First incidence (days)	671	666	699	605
Poly-3 test[d]	P=0.317N	P=0.184	P=0.413	P=0.534N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	20/50 (40%)	15/50 (30%)	16/50 (32%)	16/50 (32%)
Adjusted rate	44.9%	33.7%	34.5%	38.1%
Terminal rate	12/30 (40%)	9/30 (30%)	13/37 (35%)	7/23 (30%)
First incidence (days)	440	589	652	551
Poly-3 test	P=0.356N	P=0.191N	P=0.210N	P=0.335N
Skin (Subcutaneous Tissue): Squamous Cell Papilloma, Basal Cell Papilloma, Basal Cell Adenoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.7%	4.6%	6.5%	5.1%
Terminal rate	2/30 (7%)	1/30 (3%)	0/37 (0%)	2/23 (9%)
First incidence (days)	727 (T)	699	633	727 (T)
Poly-3 test	P=0.530	P=0.686N	P=0.541	P=0.670
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	2/50 (4%)	6/50 (12%)	2/50 (4%)	7/50 (14%)
Adjusted rate	4.7%	13.7%	4.3%	17.1%
Terminal rate	1/30 (3%)	2/30 (7%)	1/37 (3%)	2/23 (9%)
First incidence (days)	617	617	533	605
Poly-3 test	P=0.096	P=0.140	P=0.666N	P=0.067
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	3/50 (6%)	6/50 (12%)	4/50 (8%)	8/50 (16%)
Adjusted rate	7.0%	13.7%	8.7%	19.4%
Terminal rate	2/30 (7%)	2/30 (7%)	3/37 (8%)	2/23 (9%)
First incidence (days)	617	617	533	605
Poly-3 test	P=0.087	P=0.254	P=0.543	P=0.084
Testes: Adenoma				
Overall rate	45/50 (90%)	44/50 (88%)	46/50 (92%)	46/50 (92%)
Adjusted rate	95.0%	90.7%	94.9%	98.4%
Terminal rate	30/30 (100%)	29/30 (97%)	36/37 (97%)	23/23 (100%)
First incidence (days)	464	527	533	461
Poly-3 test	P=0.151	P=0.321N	P=0.698N	P=0.326
Thyroid Gland (C-Cell): Adenoma				
Overall rate	11/50 (22%)	8/50 (16%)	6/50 (12%)	5/50 (10%)
Adjusted rate	25.4%	18.1%	13.1%	12.6%
Terminal rate	8/30 (27%)	3/30 (10%)	5/37 (14%)	4/23 (17%)
First incidence (days)	533	576	681	699
Poly-3 test	P=0.079N	P=0.283N	P=0.112N	P=0.113N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	11/50 (22%)	8/50 (16%)	7/50 (14%)	5/50 (10%)
Adjusted rate	25.4%	18.1%	15.3%	12.6%
Terminal rate	8/30 (27%)	3/30 (10%)	6/37 (16%)	4/23 (17%)
First incidence (days)	533	576	681	699
Poly-3 test	P=0.090N	P=0.283N	P=0.177N	P=0.113N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
All Organs: Mononuclear Cell Leukemia				
Overall rate	18/50 (36%)	25/50 (50%)	15/50 (30%)	12/50 (24%)
Adjusted rate	39.5%	52.4%	32.2%	29.2%
Terminal rate	9/30 (30%)	12/30 (40%)	10/37 (27%)	4/23 (17%)
First incidence (days)	533	527	609	624
Poly-3 test	P=0.066N	P=0.145	P=0.303N	P=0.216N
All Organs: Malignant Mesothelioma				
Overall rate	2/50 (4%)	3/50 (6%)	4/50 (8%)	2/50 (4%)
Adjusted rate	4.6%	6.8%	8.6%	5.0%
Terminal rate	0/30 (0%)	1/30 (3%)	2/37 (5%)	1/23 (4%)
First incidence (days)	542	539	609	671
Poly-3 test	P=0.548	P=0.512	P=0.370	P=0.665
All Organs: Benign Neoplasms				
Overall rate	49/50 (98%)	49/50 (98%)	49/50 (98%)	47/50 (94%)
Adjusted rate	99.7%	99.6%	100.0%	99.7%
Terminal rate	30/30 (100%)	30/30 (100%)	37/37 (100%)	23/23 (100%)
First incidence (days)	440	527	533	461
Poly-3 test	P=0.976	P=1.000	P=1.000	P=1.000
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	28/50 (56%)	26/50 (52%)	18/50 (36%)
Adjusted rate	54.6%	56.9%	55.1%	42.4%
Terminal rate	12/30 (40%)	12/30 (40%)	18/37 (49%)	5/23 (22%)
First incidence (days)	464	435	609	570
Poly-3 test	P=0.125N	P=0.491	P=0.563	P=0.170N
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	50/50 (100%)	49/50 (98%)	48/50 (96%)
Adjusted rate	99.7%	100.0%	100.0%	99.8%
Terminal rate	30/30 (100%)	30/30 (100%)	37/37 (100%)	23/23 (100%)
First incidence (days)	440	435	533	259
Poly-3 test	P=0.999	P=1.000	P=1.000	P=1.000

(T) Terminal sacrifice

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

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

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Ginseng[a]

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Disposition Summary								
Animals initially in study	50		50		50		50	
Early deaths								
Accidental deaths	2							
Moribund	13		16		7		16	
Natural deaths	5		4		6		11	
Survivors								
Terminal sacrifice	30		30		37		23	
Animals examined microscopically	50		50		50		50	
Alimentary System								
Esophagus	(50)		(50)		(50)		(50)	
Perforation	1	(2%)						
Muscularis, degeneration	1	(2%)						
Muscularis, hemorrhage	1	(2%)						
Muscularis, inflammation	1	(2%)						
Periesophageal tissue, foreign body	1	(2%)						
Periesophageal tissue, inflammation	1	(2%)						
Intestine large, colon	(50)		(50)		(50)		(50)	
Parasite metazoan	12	(24%)	10	(20%)	11	(22%)	2	(4%)
Intestine large, rectum	(50)		(50)		(50)		(50)	
Inflammation	1	(2%)			1	(2%)		
Intestine small, ileum	(50)		(50)		(50)		(50)	
Inflammation, histiocytic					1	(2%)		
Intestine small, jejunum	(50)		(50)		(50)		(50)	
Liver	(50)		(50)		(50)		(50)	
Angiectasis	2	(4%)	6	(12%)			1	(2%)
Basophilic focus	21	(42%)	23	(46%)	27	(54%)	20	(40%)
Clear cell focus	28	(56%)	22	(44%)	25	(50%)	20	(40%)
Degeneration, cystic	9	(18%)	7	(14%)	5	(10%)	5	(10%)
Eosinophilic focus	8	(16%)	7	(14%)	5	(10%)	8	(16%)
Fatty change, focal							2	(4%)
Fatty change, diffuse	4	(8%)	3	(6%)			1	(2%)
Hematopoietic cell proliferation	2	(4%)	6	(12%)	3	(6%)	3	(6%)
Hepatodiaphragmatic nodule	3	(6%)	6	(12%)	1	(2%)	4	(8%)
Inflammation	35	(70%)	34	(68%)	41	(82%)	32	(64%)
Mixed cell focus	4	(8%)	1	(2%)	1	(2%)	5	(10%)
Necrosis	6	(12%)	4	(8%)	1	(2%)	2	(4%)
Pigmentation, hemosiderin	1	(2%)	4	(8%)				
Bile duct, cyst					1	(2%)		
Bile duct, hyperplasia	47	(94%)	49	(98%)	44	(88%)	39	(78%)
Centrilobular, degeneration	3	(6%)	2	(4%)	1	(2%)		
Hepatocyte, hyperplasia	1	(2%)	1	(2%)			1	(2%)
Oval cell, hyperplasia							1	(2%)
Serosa, fibrosis					1	(2%)		
Mesentery	(13)		(9)		(6)		(5)	
Fat, necrosis	11	(85%)	8	(89%)	4	(67%)	4	(80%)
Oral mucosa	(0)		(1)		(1)		(1)	
Pancreas	(50)		(50)		(50)		(50)	
Atrophy	24	(48%)	22	(44%)	23	(46%)	22	(44%)
Inflammation	1	(2%)	2	(4%)				
Acinus, basophilic focus							1	(2%)
Acinus, hyperplasia	1	(2%)			2	(4%)	3	(6%)
Duct, cyst			1	(2%)				
Salivary glands	(50)		(50)		(50)		(50)	
Inflammation					1	(2%)		
Necrosis					1	(2%)		
Duct, metaplasia, squamous					1	(2%)		

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

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Alimentary System (continued)								
Stomach, forestomach	(50)		(50)		(50)		(50)	
Foreign body	1	(2%)						
Inflammation	5	(10%)	3	(6%)	1	(2%)	2	(4%)
Necrosis	3	(6%)	1	(2%)			1	(2%)
Epithelium, dysplasia							1	(2%)
Epithelium, hyperplasia	4	(8%)	2	(4%)			3	(6%)
Stomach, glandular	(50)		(50)		(50)		(50)	
Dysplasia							1	(2%)
Erosion	1	(2%)						
Inflammation	2	(4%)	1	(2%)				
Mineralization					1	(2%)		
Necrosis	2	(4%)	1	(2%)				
Tongue	(0)		(0)		(1)		(0)	
Epithelium, hyperplasia					1	(100%)		
Cardiovascular System								
Blood vessel	(50)		(50)		(50)		(50)	
Mineralization							1	(2%)
Carotid artery, thrombosis	1	(2%)						
Heart	(50)		(50)		(50)		(50)	
Cardiomyopathy	48	(96%)	50	(100%)	50	(100%)	49	(98%)
Fibrosis	1	(2%)						
Atrium, thrombosis	8	(16%)	2	(4%)	3	(6%)	1	(2%)
Epicardium, inflammation	1	(2%)						
Valve, thrombosis			1	(2%)				
Endocrine System								
Adrenal cortex	(50)		(50)		(50)		(50)	
Atrophy					1	(2%)		
Degeneration, cystic	1	(2%)			1	(2%)	1	(2%)
Hypertrophy	1	(2%)	2	(4%)	4	(8%)	4	(8%)
Necrosis			1	(2%)			2	(4%)
Zona fasciculata, hyperplasia	9	(18%)	6	(12%)	7	(14%)	6	(12%)
Adrenal medulla	(50)		(50)		(50)		(50)	
Hyperplasia	9	(18%)	11	(22%)	6	(12%)	11	(22%)
Infiltration cellular, lymphocyte			1	(2%)				
Islets, pancreatic	(50)		(50)		(50)		(50)	
Hyperplasia			2	(4%)	1	(2%)	1	(2%)
Parathyroid gland	(50)		(49)		(49)		(46)	
Hyperplasia	1	(2%)	3	(6%)			1	(2%)
Pituitary gland	(50)		(50)		(50)		(50)	
Atrophy					1	(2%)		
Fibrosis							1	(2%)
Necrosis					1	(2%)		
Pars distalis, angiectasis	2	(4%)	3	(6%)	1	(2%)	1	(2%)
Pars distalis, hyperplasia	16	(32%)	20	(40%)	18	(36%)	21	(42%)
Thyroid gland	(50)		(50)		(50)		(50)	
C-cell, angiectasis	1	(2%)						
C-cell, hyperplasia	14	(28%)	14	(28%)	14	(28%)	14	(28%)
Follicular cell, hyperplasia	1	(2%)	1	(2%)				
General Body System								
None								

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Genital System								
Epididymis	(50)		(50)		(50)		(50)	
Inflammation	1	(2%)			1	(2%)		
Preputial gland	(50)		(50)		(50)		(50)	
Fibrosis			1	(2%)				
Inflammation	1	(2%)	1	(2%)	1	(2%)	2	(4%)
Prostate	(50)		(50)		(50)		(50)	
Inflammation	26	(52%)	29	(58%)	20	(40%)	21	(42%)
Epithelium, hyperplasia	3	(6%)	7	(14%)	8	(16%)	4	(8%)
Seminal vesicle	(50)		(50)		(50)		(50)	
Hyperplasia	1	(2%)						
Inflammation	1	(2%)					1	(2%)
Testes	(50)		(50)		(50)		(50)	
Germinal epithelium, degeneration							1	(2%)
Interstitial cell, hyperplasia	13	(26%)	12	(24%)	11	(22%)	9	(18%)
Hematopoietic System								
Bone marrow	(50)		(50)		(50)		(50)	
Hyperplasia	25	(50%)	28	(56%)	27	(54%)	28	(56%)
Myelofibrosis	1	(2%)	1	(2%)	1	(2%)		
Necrosis	1	(2%)						
Thrombosis	1	(2%)						
Lymph node	(7)		(15)		(4)		(6)	
Mediastinal, ectasia	2	(29%)						
Mediastinal, hemorrhage			1	(7%)				
Mediastinal, hyperplasia, lymphoid	1	(14%)					1	(17%)
Mediastinal, infiltration cellular, histiocyte			1	(7%)				
Mediastinal, infiltration cellular, plasma cell	1	(14%)	3	(20%)	1	(25%)		
Mediastinal, necrosis					1	(25%)		
Pancreatic, atrophy			1	(7%)				
Pancreatic, ectasia			2	(13%)	1	(25%)		
Lymph node, mesenteric	(49)		(50)		(50)		(50)	
Atrophy	3	(6%)	6	(12%)	3	(6%)	4	(8%)
Ectasia	1	(2%)	2	(4%)				
Hyperplasia, lymphoid							1	(2%)
Spleen	(50)		(50)		(50)		(50)	
Angiectasis			1	(2%)				
Fibrosis	1	(2%)	1	(2%)			1	(2%)
Hematopoietic cell proliferation	29	(58%)	26	(52%)	33	(66%)	26	(52%)
Hyperplasia, histiocytic			2	(4%)				
Necrosis			2	(4%)	1	(2%)	1	(2%)
Pigmentation, hemosiderin	30	(60%)	30	(60%)	31	(62%)	31	(62%)
Thrombosis	1	(2%)						
Lymphoid follicle, hyperplasia			1	(2%)				
Thymus	(50)		(47)		(49)		(50)	
Atrophy	48	(96%)	46	(98%)	47	(96%)	46	(92%)
Inflammation	1	(2%)						
Integumentary System								
Mammary gland	(50)		(50)		(50)		(50)	
Cyst					1	(2%)	1	(2%)
Skin	(50)		(50)		(50)		(50)	
Ulcer	1	(2%)	1	(2%)				
Epidermis, cyst epithelial inclusion	2	(4%)	5	(10%)	6	(12%)	7	(14%)
Epidermis, hyperplasia	2	(4%)						
Subcutaneous tissue, inflammation, histiocyte	1	(2%)						

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Musculoskeletal System								
Bone	(50)		(50)		(50)		(50)	
Cranium, osteopetrosis	1	(2%)						
Femur, osteopetrosis	1	(2%)	2	(4%)	1	(2%)	2	(4%)
Skeletal muscle	(0)		(0)		(0)		(1)	
Nervous System								
Brain	(50)		(50)		(50)		(50)	
Gliosis, focal			1	(2%)				
Necrosis			1	(2%)	2	(4%)		
Peripheral nerve	(0)		(0)		(2)		(0)	
Infiltration cellular, mononuclear cell					1	(50%)		
Respiratory System								
Larynx	(1)		(0)		(0)		(0)	
Inflammation	1	(100%)						
Lung	(50)		(50)		(50)		(50)	
Fibrosis			2	(4%)	1	(2%)	2	(4%)
Hemorrhage			1	(2%)			1	(2%)
Inflammation	4	(8%)	8	(16%)	3	(6%)	8	(16%)
Inflammation, chronic active	1	(2%)						
Thrombosis	3	(6%)						
Alveolar epithelium, hyperplasia	10	(20%)	7	(14%)	7	(14%)	6	(12%)
Alveolar epithelium, metaplasia					1	(2%)	1	(2%)
Alveolar epithelium, metaplasia, squamous	2	(4%)			2	(4%)		
Alveolus, infiltration cellular, histiocyte	15	(30%)	14	(28%)	18	(36%)	19	(38%)
Arteriole, hypertrophy	1	(2%)						
Nose	(50)		(50)		(50)		(50)	
Foreign body	7	(14%)	7	(14%)	9	(18%)	5	(10%)
Thrombosis	1	(2%)						
Nasolacrimal duct, foreign body	1	(2%)						
Nasolacrimal duct, inflammation	1	(2%)						
Olfactory epithelium, inflammation			2	(4%)	2	(4%)	4	(8%)
Respiratory epithelium, hyperplasia			1	(2%)				
Respiratory epithelium, inflammation	12	(24%)	11	(22%)	10	(20%)	16	(32%)
Trachea	(50)		(50)		(50)		(50)	
Inflammation							1	(2%)
Ulcer							1	(2%)
Epithelium, metaplasia, squamous	1	(2%)					1	(2%)
Special Senses System								
Eye	(50)		(50)		(50)		(50)	
Anterior chamber, posterior chamber, fibrosis					1	(2%)		
Cornea, inflammation	1	(2%)					1	(2%)
Lens, cataract	2	(4%)	1	(2%)			1	(2%)
Retina, degeneration	4	(8%)					3	(6%)
Retina, fibrosis	1	(2%)						
Retina, mineralization	1	(2%)						
Retina, necrosis							1	(2%)
Harderian gland	(50)		(50)		(50)		(50)	
Metaplasia			1	(2%)	1	(2%)		
Zymbal's gland	(0)		(0)		(0)		(1)	

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Urinary System								
Kidney	(50)		(50)		(50)		(50)	
Accumulation, hyaline droplet	2	(4%)	2	(4%)			1	(2%)
Amyloid deposition			1	(2%)				
Mineralization	26	(52%)	31	(62%)	29	(58%)	35	(70%)
Nephropathy	47	(94%)	50	(100%)	50	(100%)	44	(88%)
Cortex, cyst			1	(2%)				
Cortex, hemorrhage							1	(2%)
Papilla, necrosis							1	(2%)
Papilla, transitional epithelium, hyperplasia	1	(2%)	1	(2%)				
Renal tubule, dilatation							1	(2%)
Renal tubule, hyperplasia, atypical	1	(2%)						
Urinary bladder	(50)		(50)		(50)		(50)	
Inflammation							1	(2%)

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

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

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			3	1
Moribund	8	12	7	10
Natural deaths	6	11	6	15
Survivors				
Terminal sacrifice	36	27	34	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Squamous cell papilloma				1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Leiomyoma	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Leiomyoma		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Hepatocellular carcinoma		1 (2%)		
Mesentery	(9)	(8)	(6)	(6)
Schwannoma malignant		1 (13%)		
Oral mucosa	(0)	(0)	(1)	(0)
Pharyngeal, squamous cell papilloma			1 (100%)	
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(0)	(0)	(0)
Tooth	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	1 (2%)			1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Parathyroid gland	(48)	(45)	(46)	(50)
Pituitary gland	(50)	(49)	(50)	(50)
Pars distalis, adenoma	16 (32%)	15 (31%)	13 (26%)	11 (22%)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma		2 (4%)		
C-cell, adenoma	6 (12%)	5 (10%)	5 (10%)	3 (6%)
C-cell, carcinoma				1 (2%)
General Body System				
None				

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Genital System								
Clitoral gland	(50)		(50)		(50)		(50)	
Adenoma	1	(2%)			4	(8%)		
Carcinoma	1	(2%)						
Ovary	(50)		(50)		(50)		(50)	
Hemangiosarcoma			1	(2%)				
Uterus	(50)		(50)		(50)		(50)	
Polyp stromal	7	(14%)	3	(6%)	7	(14%)	5	(10%)
Polyp stromal, multiple	1	(2%)					1	(2%)
Vagina	(0)		(0)		(2)		(0)	
Polyp					1	(50%)		
Hematopoietic System								
Bone marrow	(50)		(50)		(50)		(50)	
Lymph node	(1)		(5)		(4)		(4)	
Mediastinal, carcinoma, metastatic, thyroid gland							1	(25%)
Lymph node, mesenteric	(50)		(50)		(50)		(50)	
Spleen	(50)		(50)		(50)		(50)	
Thymus	(48)		(49)		(50)		(50)	
Thymoma malignant	1	(2%)						
Integumentary System								
Mammary gland	(50)		(50)		(50)		(50)	
Adenoma	2	(4%)					1	(2%)
Carcinoma			3	(6%)				
Fibroadenoma	24	(48%)	18	(36%)	22	(44%)	11	(22%)
Fibroadenoma, multiple	8	(16%)	12	(24%)	8	(16%)	5	(10%)
Skin	(50)		(50)		(50)		(50)	
Epidermis, basal cell adenoma	1	(2%)						
Epidermis, keratoacanthoma	2	(4%)						
Epidermis, squamous cell papilloma	1	(2%)			1	(2%)		
Subcutaneous tissue, fibroma					2	(4%)		
Musculoskeletal System								
Bone	(50)		(50)		(50)		(50)	
Skeletal muscle	(1)		(1)		(0)		(0)	
Nervous System								
Brain	(50)		(50)		(50)		(50)	
Astrocytoma benign			1	(2%)				
Peripheral nerve	(1)		(1)		(0)		(0)	
Spinal cord	(1)		(1)		(0)		(0)	
Glioma malignant	1	(100%)						
Respiratory System								
Larynx	(1)		(0)		(0)		(2)	
Lung	(50)		(50)		(50)		(50)	
Alveolar/bronchiolar adenoma	1	(2%)						
Carcinoma, metastatic, mammary gland			1	(2%)				
Nose	(50)		(50)		(50)		(50)	
Adenoma	1	(2%)						

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Iris, melanoma malignant	1 (2%)			1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Lipoma		1 (2%)		1 (2%)
Urethra	(0)	(1)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	6 (12%)	9 (18%)	8 (16%)	9 (18%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	45	40	40	34
Total primary neoplasms	84	74	73	52
Total animals with benign neoplasms	42	34	39	28
Total benign neoplasms	74	59	65	41
Total animals with malignant neoplasms	10	14	8	11
Total malignant neoplasms	10	15	8	11
Total animals with metastatic neoplasms		1		1
Total metastatic neoplasms		1		1

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

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Clitoral Gland: Adenoma				
Overall rate[a]	1/50 (2%)	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rate[b]	2.2%	0.0%	9.5%	0.0%
Terminal rate[c]	1/36 (3%)	0/27 (0%)	4/34 (12%)	0/24 (0%)
First incidence (days)	728 (T)	—[e]	728 (T)	—
Poly-3 test[d]	P=0.585N	P=0.527N	P=0.157	P=0.529N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rate	4.4%	0.0%	9.5%	0.0%
Terminal rate	2/36 (6%)	0/27 (0%)	4/34 (12%)	0/24 (0%)
First incidence (days)	728 (T)	—	728 (T)	—
Poly-3 test	P=0.378N	P=0.268N	P=0.304	P=0.271N
Mammary Gland: Fibroadenoma				
Overall rate	32/50 (64%)	30/50 (60%)	30/50 (60%)	16/50 (32%)
Adjusted rate	69.2%	71.0%	69.1%	38.4%
Terminal rate	27/36 (75%)	21/27 (78%)	24/34 (71%)	7/24 (29%)
First incidence (days)	668	533	643	461
Poly-3 test	P<0.001N	P=0.518	P=0.589N	P=0.002N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	33/50 (66%)	30/50 (60%)	30/50 (60%)	17/50 (34%)
Adjusted rate	71.4%	71.0%	69.1%	40.8%
Terminal rate	28/36 (78%)	21/27 (78%)	24/34 (71%)	8/24 (33%)
First incidence (days)	668	533	643	461
Poly-3 test	P<0.001N	P=0.584N	P=0.498N	P=0.002N
Mammary Gland: Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	7.3%	0.0%	0.0%
Terminal rate	0/36 (0%)	0/27 (0%)	0/34 (0%)	0/24 (0%)
First incidence (days)	—	533	—	—
Poly-3 test	P=0.361N	P=0.102	—[f]	—
Mammary Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	4.4%	7.3%	0.0%	2.6%
Terminal rate	2/36 (6%)	0/27 (0%)	0/34 (0%)	1/24 (4%)
First incidence (days)	728 (T)	533	—	728 (T)
Poly-3 test	P=0.271N	P=0.455	P=0.254N	P=0.551N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	33/50 (66%)	31/50 (62%)	30/50 (60%)	17/50 (34%)
Adjusted rate	71.4%	72.4%	69.1%	40.8%
Terminal rate	28/36 (78%)	21/27 (78%)	24/34 (71%)	8/24 (33%)
First incidence (days)	668	533	643	461
Poly-3 test	P<0.001N	P=0.553	P=0.498N	P=0.002N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	16/50 (32%)	15/49 (31%)	13/50 (26%)	11/50 (22%)
Adjusted rate	34.0%	36.3%	29.5%	26.7%
Terminal rate	10/36 (28%)	10/27 (37%)	8/34 (24%)	6/24 (25%)
First incidence (days)	592	533	459	541
Poly-3 test	P=0.216N	P=0.500	P=0.406N	P=0.306N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	3/50 (6%)[g]	0/50 (0%)	1/50 (2%)	0/50 (0%)
Adjusted rate	6.6%	0.0%	2.3%	0.0%
Terminal rate	3/36 (8%)	0/27 (0%)	0/34 (0%)	0/24 (0%)
First incidence (days)	728 (T)	—	533	—
Poly-3 test	P=0.083N	P=0.144N	P=0.326N	P=0.147N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	6/50 (12%)	7/50 (14%)	5/50 (10%)	3/50 (6%)
Adjusted rate	13.2%	17.5%	11.8%	7.6%
Terminal rate	6/36 (17%)	6/27 (22%)	4/34 (12%)	2/24 (8%)
First incidence (days)	728 (T)	618	646	541
Poly-3 test	P=0.196N	P=0.405	P=0.546N	P=0.310N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	6/50 (12%)	7/50 (14%)	5/50 (10%)	4/50 (8%)
Adjusted rate	13.2%	17.5%	11.8%	10.0%
Terminal rate	6/36 (17%)	6/27 (22%)	4/34 (12%)	2/24 (8%)
First incidence (days)	728 (T)	618	646	541
Poly-3 test	P=0.306N	P=0.405	P=0.546N	P=0.448N
Uterus: Stromal Polyp				
Overall rate	8/50 (16%)	3/50 (6%)	7/50 (14%)	6/50 (12%)
Adjusted rate	17.3%	7.5%	16.2%	15.1%
Terminal rate	5/36 (14%)	2/27 (7%)	5/34 (15%)	5/24 (21%)
First incidence (days)	561	598	497	572
Poly-3 test	P=0.542	P=0.150N	P=0.560N	P=0.510N
All Organs: Mononuclear Cell Leukemia				
Overall rate	6/50 (12%)	9/50 (18%)	8/50 (16%)	9/50 (18%)
Adjusted rate	13.1%	21.6%	18.9%	22.1%
Terminal rate	4/36 (11%)	4/27 (15%)	7/34 (21%)	3/24 (13%)
First incidence (days)	659	500	646	600
Poly-3 test	P=0.214	P=0.220	P=0.328	P=0.206
All Organs: Benign Neoplasms				
Overall rate	42/50 (84%)	34/50 (68%)	39/50 (78%)	28/50 (56%)
Adjusted rate	87.1%	79.8%	84.5%	62.9%
Terminal rate	32/36 (89%)	24/27 (89%)	29/34 (85%)	12/24 (50%)
First incidence (days)	561	533	459	461
Poly-3 test	P=0.002N	P=0.238N	P=0.475N	P=0.004N
All Organs: Malignant Neoplasms				
Overall rate	10/50 (20%)	14/50 (28%)	8/50 (16%)	11/50 (22%)
Adjusted rate	21.5%	32.3%	18.9%	26.8%
Terminal rate	6/36 (17%)	5/27 (19%)	7/34 (21%)	4/24 (17%)
First incidence (days)	561	500	646	600
Poly-3 test	P=0.472	P=0.179	P=0.481N	P=0.373

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	45/50 (90%)	40/50 (80%)	40/50 (80%)	34/50 (68%)
Adjusted rate	92.4%	88.1%	86.7%	74%
Terminal rate	33/36 (92%)	24/27 (89%)	30/34 (88%)	14/24 (58%)
First incidence (days)	561	500	459	461
Poly-3 test	P=0.005N	P=0.352N	P=0.275N	P=0.012

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for clitoral gland, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. 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.
[g] One basal cell adenoma occurred in an animal that also had a keratoacanthoma

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Ginseng[a]

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Disposition Summary								
Animals initially in study	50		50		50		50	
Early deaths								
Accidental deaths					3		1	
Moribund	8		12		7		10	
Natural deaths	6		11		6		15	
Survivors								
Terminal sacrifice	36		27		34		24	
Animals examined microscopically	50		50		50		50	
Alimentary System								
Esophagus	(50)		(50)		(50)		(50)	
Perforation							1	(2%)
Muscularis, degeneration			1	(2%)				
Periesophageal tissue, foreign body							1	(2%)
Periesophageal tissue, inflammation							1	(2%)
Periesophageal tissue, necrosis							1	(2%)
Intestine large, cecum	(50)		(50)		(50)		(50)	
Inflammation			1	(2%)				
Intestine large, colon	(50)		(50)		(50)		(50)	
Parasite metazoan	5	(10%)	9	(18%)	7	(14%)	4	(8%)
Intestine large, rectum	(50)		(50)		(50)		(50)	
Intestine small, duodenum	(50)		(50)		(50)		(50)	
Intestine small, jejunum	(50)		(50)		(50)		(50)	
Liver	(50)		(50)		(50)		(50)	
Angiectasis	1	(2%)	1	(2%)			2	(4%)
Basophilic focus	46	(92%)	38	(76%)	46	(92%)	45	(90%)
Clear cell focus	6	(12%)	3	(6%)	8	(16%)	6	(12%)
Cytoplasmic alteration, focal					1	(2%)		
Degeneration, cystic	1	(2%)						
Eosinophilic focus	16	(32%)	10	(20%)	12	(24%)	13	(26%)
Fatty change, focal	1	(2%)	2	(4%)				
Fatty change, diffuse	5	(10%)	4	(8%)	4	(8%)	5	(10%)
Hematopoietic cell proliferation	6	(12%)	6	(12%)	7	(14%)	4	(8%)
Hepatodiaphragmatic nodule	4	(8%)	4	(8%)	2	(4%)	5	(10%)
Inflammation	43	(86%)	38	(76%)	43	(86%)	45	(90%)
Mixed cell focus	5	(10%)	3	(6%)	5	(10%)	4	(8%)
Necrosis			2	(4%)	1	(2%)	1	(2%)
Pigmentation, hemosiderin	2	(4%)	3	(6%)			2	(4%)
Tension lipidosis					1	(2%)	1	(2%)
Bile duct, hyperplasia	19	(38%)	19	(38%)	17	(34%)	9	(18%)
Centrilobular, degeneration			1	(2%)	2	(4%)		
Oval cell, hyperplasia					2	(4%)		
Mesentery	(9)		(8)		(6)		(6)	
Fat, necrosis	9	(100%)	8	(100%)	6	(100%)	6	(100%)
Oral mucosa	(0)		(0)		(1)		(0)	
Pancreas	(50)		(50)		(50)		(50)	
Atrophy	16	(32%)	11	(22%)	12	(24%)	12	(24%)
Basophilic focus					1	(2%)		
Inflammation	1	(2%)						
Acinus, vacuolization cytoplasmic	1	(2%)						
Salivary glands	(50)		(50)		(50)		(50)	
Atrophy	1	(2%)						
Inflammation	1	(2%)						

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

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Alimentary System (continued)								
Stomach, forestomach	(50)		(50)		(50)		(50)	
Inflammation	1	(2%)	2	(4%)				
Mineralization							1	(2%)
Necrosis	1	(2%)						
Epithelium, cyst							1	(2%)
Epithelium, dysplasia			1	(2%)				
Epithelium, hyperplasia	1	(2%)	2	(4%)			3	(6%)
Stomach, glandular	(50)		(50)		(50)		(50)	
Inflammation							1	(2%)
Mineralization							1	(2%)
Tongue	(1)		(0)		(0)		(0)	
Epithelium, hyperplasia	1	(100%)						
Tooth	(1)		(0)		(0)		(0)	
Peridontal tissue, inflammation	1	(100%)						
Cardiovascular System								
Blood vessel	(50)		(50)		(50)		(50)	
Inflammation	1	(2%)	1	(2%)			1	(2%)
Mineralization							1	(2%)
Heart	(50)		(50)		(50)		(50)	
Cardiomyopathy	47	(94%)	43	(86%)	48	(96%)	46	(92%)
Mineralization							1	(2%)
Atrium, thrombosis			1	(2%)			1	(2%)
Endocardium, hyperplasia	1	(2%)						
Endocrine System								
Adrenal cortex	(50)		(50)		(50)		(50)	
Atrophy	1	(2%)						
Degeneration, cystic	5	(10%)	6	(12%)	4	(8%)	4	(8%)
Hypertrophy	3	(6%)	6	(12%)	1	(2%)	2	(4%)
Necrosis	2	(4%)					2	(4%)
Zona fasciculata, hyperplasia	11	(22%)	10	(20%)	7	(14%)	8	(16%)
Adrenal medulla	(50)		(50)		(50)		(50)	
Hyperplasia	2	(4%)	3	(6%)	3	(6%)	1	(2%)
Islets, pancreatic	(50)		(50)		(50)		(50)	
Atrophy	1	(2%)						
Hyperplasia					1	(2%)		
Inflammation, chronic	1	(2%)						
Parathyroid gland	(48)		(45)		(46)		(50)	
Hyperplasia							1	(2%)
Pituitary gland	(50)		(49)		(50)		(50)	
Hemorrhage	1	(2%)					1	(2%)
Pars distalis, angiectasis	7	(14%)	2	(4%)	6	(12%)	3	(6%)
Pars distalis, cyst			1	(2%)			2	(4%)
Pars distalis, hyperplasia	23	(46%)	19	(39%)	19	(38%)	20	(40%)
Pars intermedia, hyperplasia					1	(2%)		
Thyroid gland	(50)		(50)		(50)		(50)	
C-cell, hyperplasia	18	(36%)	18	(36%)	17	(34%)	19	(38%)
General Body System								
None								

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Genital System								
Clitoral gland	(50)		(50)		(50)		(50)	
Inflammation			1	(2%)	1	(2%)	1	(2%)
Ovary	(50)		(50)		(50)		(50)	
Cyst	6	(12%)	5	(10%)	9	(18%)	7	(14%)
Uterus	(50)		(50)		(50)		(50)	
Inflammation			1	(2%)				
Thrombosis			1	(2%)				
Cervix, cyst, multiple			1	(2%)				
Endometrium, hyperplasia, cystic	4	(8%)	5	(10%)	3	(6%)	4	(8%)
Vagina	(0)		(0)		(2)		(0)	
Inflammation					1	(50%)		
Hematopoietic System								
Bone marrow	(50)		(50)		(50)		(50)	
Hyperplasia	13	(26%)	18	(36%)	18	(36%)	18	(36%)
Myelofibrosis	1	(2%)					1	(2%)
Lymph node	(1)		(5)		(4)		(4)	
Deep cervical, infiltration cellular, histiocyte	1	(100%)	1	(20%)				
Deep cervical, infiltration cellular, mast cell					1	(25%)		
Mediastinal, hyperplasia, lymphoid			2	(40%)			1	(25%)
Mediastinal, infiltration cellular, histiocyte					1	(25%)		
Mediastinal, infiltration cellular, plasma cell					1	(25%)	1	(25%)
Lymph node, mesenteric	(50)		(50)		(50)		(50)	
Atrophy	2	(4%)			3	(6%)	1	(2%)
Hyperplasia, lymphoid							2	(4%)
Spleen	(50)		(50)		(50)		(50)	
Hematopoietic cell proliferation	42	(84%)	41	(82%)	42	(84%)	38	(76%)
Hyperplasia, histiocytic	1	(2%)			1	(2%)		
Pigmentation, hemosiderin	44	(88%)	41	(82%)	40	(80%)	43	(86%)
Capsule, fibrosis	1	(2%)						
Lymphoid follicle, atrophy	1	(2%)						
Red pulp, atrophy							1	(2%)
Thymus	(48)		(49)		(50)		(50)	
Atrophy	47	(98%)	44	(90%)	49	(98%)	50	(100%)
Integumentary System								
Mammary gland	(50)		(50)		(50)		(50)	
Cyst					1	(2%)		
Hyperplasia			2	(4%)				
Skin	(50)		(50)		(50)		(50)	
Ulcer							1	(2%)
Epidermis, atrophy							1	(2%)
Epidermis, cyst epithelial inclusion			1	(2%)				
Subcutaneous tissue, inflammation							1	(2%)
Musculoskeletal System								
Bone	(50)		(50)		(50)		(50)	
Femur, osteopetrosis	3	(6%)						
Skeletal muscle	(1)		(1)		(0)		(0)	
Atrophy	1	(100%)						

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Nervous System								
Brain	(50)		(50)		(50)		(50)	
Gliosis			1	(2%)				
Infiltration cellular, mononuclear cell	1	(2%)	1	(2%)			1	(2%)
Cerebrum, necrosis	1	(2%)						
Peripheral nerve	(1)		(1)		(0)		(0)	
Radial, ulnar, degeneration	1	(100%)						
Spinal cord	(1)		(1)		(0)		(0)	
Respiratory System								
Larynx	(1)		(0)		(0)		(2)	
Foreign body	1	(100%)					2	(100%)
Lung	(50)		(50)		(50)		(50)	
Congestion					2	(4%)	1	(2%)
Fibrosis	1	(2%)					1	(2%)
Hemorrhage							1	(2%)
Inflammation	4	(8%)	7	(14%)	6	(12%)	5	(10%)
Mineralization							1	(2%)
Alveolar epithelium, hyperplasia	10	(20%)	10	(20%)	6	(12%)	5	(10%)
Alveolar epithelium, metaplasia	1	(2%)	1	(2%)				
Alveolar epithelium, metaplasia, squamous			1	(2%)	1	(2%)	1	(2%)
Alveolus, infiltration cellular, histiocyte	14	(28%)	13	(26%)	15	(30%)	9	(18%)
Nose	(50)		(50)		(50)		(50)	
Foreign body	1	(2%)	1	(2%)	1	(2%)	2	(4%)
Thrombosis	1	(2%)						
Nasolacrimal duct, inflammation			1	(2%)				
Olfactory epithelium, hyperplasia			1	(2%)				
Olfactory epithelium, inflammation	1	(2%)					4	(8%)
Respiratory epithelium, hyperplasia	1	(2%)	1	(2%)				
Respiratory epithelium, inflammation	3	(6%)	2	(4%)	1	(2%)	10	(20%)
Special Senses System								
Eye	(50)		(50)		(50)		(50)	
Fibrosis	1	(2%)						
Synechia					1	(2%)		
Cornea, fibrosis			3	(6%)	1	(2%)	2	(4%)
Cornea, hyperplasia, squamous					1	(2%)		
Cornea, inflammation			1	(2%)			2	(4%)
Cornea, pigmentation, hemosiderin							1	(2%)
Lens, cataract	3	(6%)	3	(6%)	4	(8%)	2	(4%)
Optic nerve, degeneration	1	(2%)			1	(2%)		
Retina, degeneration	3	(6%)	4	(8%)	2	(4%)	1	(2%)
Retina, gliosis							1	(2%)
Harderian gland	(50)		(50)		(50)		(50)	
Atrophy							1	(2%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Urinary System								
Kidney	(50)		(50)		(50)		(50)	
Accumulation, hyaline droplet	1	(2%)						
Infarct			1	(2%)	1	(2%)		
Mineralization	39	(78%)	37	(74%)	41	(82%)	46	(92%)
Nephropathy	42	(84%)	36	(72%)	43	(86%)	39	(78%)
Papilla, necrosis	1	(2%)					1	(2%)
Papilla, transitional epithelium, hyperplasia	1	(2%)			1	(2%)		
Pelvis, inflammation	3	(6%)					1	(2%)
Pelvis, transitional epithelium, hyperplasia	1	(2%)						
Renal tubule, hyperplasia, atypical	1	(2%)					1	(2%)
Renal tubule, infarct	1	(2%)					1	(2%)
Urethra	(0)		(1)		(0)		(0)	
Inflammation			1	(100%)				
Urinary bladder	(50)		(50)		(50)		(50)	
Calculus gross observation	2	(4%)						
Hemorrhage			1	(2%)				
Inflammation			1	(2%)				
Mineralization							1	(2%)
Transitional epithelium, hyperplasia			1	(2%)				
Transitional epithelium, hyperplasia, adenomatous	2	(4%)						

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

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

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Disposition Summary								
Animals initially in study	50		50		50		50	
Early deaths								
Accidental death							1	
Moribund	12		10		8		8	
Natural deaths	6		7		4		8	
Survivors								
Died last week of study			1				1	
Terminal sacrifice	32		32		38		32	
Animals examined microscopically	50		50		50		50	
Alimentary System								
Esophagus	(50)		(50)		(50)		(50)	
Gallbladder	(49)		(50)		(49)		(50)	
Intestine large, cecum	(50)		(50)		(50)		(50)	
Adenoma							1	(2%)
Intestine large, colon	(50)		(50)		(50)		(50)	
Intestine small, duodenum	(50)		(50)		(50)		(50)	
Adenoma			1	(2%)	1	(2%)		
Intestine small, ileum	(50)		(50)		(50)		(50)	
Intestine small, jejunum	(50)		(50)		(50)		(50)	
Adenoma							1	(2%)
Carcinoma			1	(2%)			2	(4%)
Hemangiosarcoma							1	(2%)
Liver	(50)		(50)		(50)		(50)	
Carcinoma, metastatic, uncertain primary site							1	(2%)
Cholangiocarcinoma			1	(2%)	1	(2%)		
Hemangioma					1	(2%)		
Hemangiosarcoma	2	(4%)	4	(8%)	1	(2%)	3	(6%)
Hepatoblastoma	1	(2%)	1	(2%)			1	(2%)
Hepatocellular adenoma	19	(38%)	11	(22%)	11	(22%)	12	(24%)
Hepatocellular adenoma, multiple	15	(30%)	18	(36%)	22	(44%)	22	(44%)
Hepatocellular carcinoma	7	(14%)	9	(18%)	10	(20%)	10	(20%)
Hepatocellular carcinoma, multiple	7	(14%)	5	(10%)	5	(10%)	2	(4%)
Hepatocholangiocarcinoma					1	(2%)		
Schwannoma malignant, metastatic, skin	1	(2%)						
Mesentery	(3)		(4)		(6)		(0)	
Cholangiocarcinoma, metastatic, liver					1	(17%)		
Hemangiosarcoma, metastatic, epididymis					1	(17%)		
Hepatocellular carcinoma, metastatic, liver	1	(33%)	1	(25%)				
Pancreas	(50)		(50)		(50)		(50)	
Salivary glands	(50)		(50)		(50)		(50)	
Mast cell tumor malignant	1	(2%)						
Stomach, forestomach	(50)		(50)		(50)		(50)	
Squamous cell papilloma	1	(2%)	1	(2%)	1	(2%)		
Stomach, glandular	(50)		(50)		(50)		(50)	
Cholangiocarcinoma, metastatic, liver					1	(2%)		
Tooth	(13)		(15)		(9)		(13)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Cardiovascular System								
Blood vessel	(50)		(48)		(50)		(50)	
Aorta, carcinoma, metastatic, uncertain primary site							1	(2%)
Heart	(50)		(50)		(50)		(50)	
Carcinoma, metastatic, uncertain primary site							1	(2%)
Cholangiocarcinoma, metastatic, liver			1	(2%)	1	(2%)		
Hemangioma							1	(2%)
Hemangiosarcoma	1	(2%)	1	(2%)				
Sarcoma, metastatic, skeletal muscle	1	(2%)						
Endocrine System								
Adrenal cortex	(50)		(50)		(50)		(50)	
Subcapsular, adenoma	1	(2%)	2	(4%)	4	(8%)	2	(4%)
Adrenal medulla	(50)		(50)		(50)		(50)	
Pheochromocytoma benign			1	(2%)			1	(2%)
Islets, pancreatic	(50)		(50)		(50)		(50)	
Adenoma	1	(2%)	1	(2%)	1	(2%)	1	(2%)
Carcinoma	1	(2%)						
Parathyroid gland	(49)		(48)		(45)		(48)	
Pituitary gland	(50)		(50)		(50)		(50)	
Pars distalis, adenoma	1	(2%)						
Pars intermedia, adenoma							1	(2%)
Thyroid gland	(50)		(50)		(50)		(50)	
Follicular cell, adenoma	1	(2%)						
General Body System								
None								
Genital System								
Epididymis	(50)		(50)		(50)		(50)	
Cholangiocarcinoma, metastatic, liver					1	(2%)		
Hemangiosarcoma					1	(2%)		
Hepatocellular carcinoma, metastatic, liver			1	(2%)				
Preputial gland	(50)		(50)		(50)		(50)	
Prostate	(50)		(50)		(50)		(50)	
Carcinoma							1	(2%)
Cholangiocarcinoma, metastatic, liver					1	(2%)		
Seminal vesicle	(50)		(50)		(50)		(50)	
Cholangiocarcinoma, metastatic, liver					1	(2%)		
Testes	(50)		(50)		(50)		(50)	
Interstitial cell, adenoma			1	(2%)	1	(2%)		
Hematopoietic System								
Bone marrow	(50)		(50)		(50)		(50)	
Hemangiosarcoma			1	(2%)				
Hemangiosarcoma, metastatic, spleen	1	(2%)						
Lymph node	(2)		(2)		(2)		(1)	
Inguinal, hepatocellular carcinoma, metastatic, liver			1	(50%)				
Mediastinal, cholangiocarcinoma, metastatic, liver					1	(50%)		
Renal, hepatocellular carcinoma, metastatic, liver			1	(50%)				

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Hematopoietic System (continued)								
Lymph node, mandibular	(50)		(50)		(50)		(50)	
Mast cell tumor malignant	1	(2%)						
Lymph node, mesenteric	(50)		(49)		(50)		(50)	
Cholangiocarcinoma, metastatic, liver			1	(2%)				
Spleen	(50)		(50)		(50)		(50)	
Hemangiosarcoma	1	(2%)	1	(2%)			1	(2%)
Thymus	(47)		(50)		(50)		(49)	
Carcinoma, metastatic, uncertain primary site							1	(2%)
Cholangiocarcinoma, metastatic, liver			1	(2%)	1	(2%)		
Sarcoma, metastatic, skeletal muscle	1	(2%)						
Integumentary System								
Skin	(50)		(50)		(50)		(50)	
Squamous cell papilloma					2	(4%)		
Subcutaneous tissue, fibrosarcoma	1	(2%)						
Subcutaneous tissue, hemangioma	1	(2%)	1	(2%)				
Subcutaneous tissue, schwannoma, malignant	1	(2%)						
Musculoskeletal System								
Skeletal muscle	(1)		(1)		(0)		(0)	
Cholangiocarcinoma, metastatic, liver			1	(100%)				
Sarcoma	1	(100%)						
Nervous System								
Brain	(50)		(50)		(50)		(50)	
Respiratory System								
Lung	(50)		(50)		(50)		(50)	
Alveolar/bronchiolar adenoma	7	(14%)	2	(4%)	5	(10%)	15	(30%)
Alveolar/bronchiolar adenoma, multiple	2	(4%)	1	(2%)	1	(2%)	1	(2%)
Alveolar/bronchiolar carcinoma	3	(6%)	3	(6%)	4	(8%)	3	(6%)
Alveolar/bronchiolar carcinoma, multiple					1	(2%)		
Carcinoma, metastatic, Harderian gland	1	(2%)						
Carcinoma, metastatic, uncertain primary site							1	(2%)
Cholangiocarcinoma, metastatic, liver			1	(2%)	1	(2%)		
Hemangiosarcoma, metastatic, liver	1	(2%)	1	(2%)			1	(2%)
Hepatoblastoma, metastatic, liver					1	(2%)		
Hepatocellular carcinoma, metastatic, liver	2	(4%)	8	(16%)	6	(12%)	4	(8%)
Sarcoma, metastatic, skeletal muscle	1	(2%)						
Serosa, cholangiocarcinoma, metastatic, liver					1	(2%)		
Nose	(50)		(49)		(49)		(50)	
Trachea	(50)		(50)		(50)		(50)	
Carcinoma, metastatic, uncertain primary site							1	(2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Special Senses System								
Eye	(50)		(50)		(50)		(50)	
Harderian gland	(50)		(50)		(50)		(50)	
Adenoma	10	(20%)	7	(14%)	11	(22%)	14	(28%)
Carcinoma	1	(2%)						
Bilateral, adenoma	1	(2%)					1	(2%)
Urinary System								
Kidney	(50)		(50)		(50)		(50)	
Adenoma							1	(2%)
Adenoma, multiple							1	(2%)
Carcinoma	1	(2%)						
Urinary bladder	(50)		(50)		(50)		(50)	
Systemic Lesions								
Multiple organs[b]	(50)		(50)		(50)		(50)	
Histiocytic sarcoma	1	(2%)			1	(2%)		
Lymphoma malignant	4	(8%)	1	(2%)	2	(4%)		
Neoplasm Summary								
Total animals with primary neoplasms[c]	46		44		46		47	
Total primary neoplasms	95		75		88		99	
Total animals with benign neoplasms	38		34		40		44	
Total benign neoplasms	60		47		61		75	
Total animals with malignant neoplasms	26		22		24		22	
Total malignant neoplasms	35		28		27		24	
Total animals with metastatic neoplasms	7		10		8		6	
Total metastatic neoplasms	10		18		18		11	
Total animals with malignant neoplasms of uncertain primary site							1	

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

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	1/50 (2%)	2/50 (4%)	4/50 (8%)	2/50 (4%)
Adjusted rate[b]	2.3%	4.5%	8.7%	4.7%
Terminal rate[c]	1/32 (3%)	2/32 (6%)	4/38 (11%)	2/32 (6%)
First incidence (days)	730 (T)	730 (T)	730 (T)	730 (T)
Poly-3 test[d]	P=0.362	P=0.503	P=0.195	P=0.487
Harderian Gland: Adenoma				
Overall rate	11/50 (22%)	7/50 (14%)	11/50 (22%)	15/50 (30%)
Adjusted rate	23.9%	15.3%	22.8%	34.1%
Terminal rate	5/32 (16%)	3/32 (9%)	5/38 (13%)	11/32 (34%)
First incidence (days)	562	542	486	483
Poly-3 test	P=0.083	P=0.220N	P=0.546N	P=0.201
Harderian Gland: Adenoma or Carcinoma				
Overall rate	12/50 (24%)	7/50 (14%)	11/50 (22%)	15/50 (30%)
Adjusted rate	26.0%	15.3%	22.8%	34.1%
Terminal rate	5/32 (16%)	3/32 (9%)	5/38 (13%)	11/32 (34%)
First incidence (days)	562	542	486	483
Poly-3 test	P=0.120	P=0.156N	P=0.452N	P=0.271
Small Intestine (Duodenum or Jejunum): Adenoma or Carcinoma				
Overall rate	0/50 (0%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	4.5%	2.2%	7.0%
Terminal rate	0/32 (0%)	2/32 (6%)	1/38 (3%)	1/32 (3%)
First incidence (days)	—[e]	730 (T)	730 (T)	617
Poly-3 test	P=0.100	P=0.239	P=0.510	P=0.115
Liver: Hemangiosarcoma				
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.6%	8.9%	2.2%	7.0%
Terminal rate	1/32 (3%)	2/32 (6%)	1/38 (3%)	1/32 (3%)
First incidence (days)	695	542	730 (T)	558
Poly-3 test	P=0.537	P=0.347	P=0.483N	P=0.490
Liver: Hepatocellular Adenoma				
Overall rate	34/50 (68%)	29/50 (58%)	33/50 (66%)	34/50 (68%)
Adjusted rate	71.6%	61.8%	69.7%	74.3%
Terminal rate	26/32 (81%)	20/32 (63%)	28/38 (74%)	23/32 (72%)
First incidence (days)	339	542	549	429
Poly-3 test	P=0.297	P=0.205N	P=0.508N	P=0.478
Liver: Hepatocellular Carcinoma				
Overall rate	14/50 (28%)	14/50 (28%)	15/50 (30%)	12/50 (24%)
Adjusted rate	31.4%	30.7%	32.1%	27.7%
Terminal rate	11/32 (34%)	9/32 (28%)	13/38 (34%)	6/32 (19%)
First incidence (days)	617	576	632	563
Poly-3 test	P=0.399N	P=0.564N	P=0.559	P=0.441N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	39/50 (78%)	38/50 (76%)	38/50 (76%)	39/50 (78%)
Adjusted rate	81.3%	79.2%	79.4%	84.8%
Terminal rate	29/32 (91%)	25/32 (78%)	31/38 (82%)	27/32 (84%)
First incidence (days)	339	542	549	429
Poly-3 test	P=0.342	P=0.495N	P=0.508N	P=0.426

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	14/50 (28%)	15/50 (30%)	15/50 (30%)	13/50 (26%)
Adjusted rate	31.4%	32.9%	32.1%	30.0%
Terminal rate	11/32 (34%)	10/32 (31%)	13/38 (34%)	7/32 (22%)
First incidence (days)	617	576	632	563
Poly-3 test	P=0.464N	P=0.527	P=0.559	P=0.535N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	39/50 (78%)	38/50 (76%)	38/50 (76%)	39/50 (78%)
Adjusted rate	81.3%	79.2%	79.4%	84.8%
Terminal rate	29/32 (91%)	25/32 (78%)	31/38 (82%)	27/32 (84%)
First incidence (days)	339	542	549	429
Poly-3 test	P=0.342	P=0.495N	P=0.508N	P=0.426
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	9/50 (18%)	3/50 (6%)	6/50 (12%)	16/50 (32%)
Adjusted rate	20.5%	6.8%	12.9%	36.0%
Terminal rate	8/32 (25%)	2/32 (6%)	5/38 (13%)	9/32 (28%)
First incidence (days)	714	715	632	558
Poly-3 test	P=0.008	P=0.056N	P=0.246N	P=0.080
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	5/50 (10%)	3/50 (6%)
Adjusted rate	6.7%	6.7%	10.8%	7.0%
Terminal rate	1/32 (3%)	1/32 (3%)	4/38 (11%)	2/32 (6%)
First incidence (days)	562	576	714	617
Poly-3 test	P=0.505	P=0.662N	P=0.375	P=0.641
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	12/50 (24%)	6/50 (12%)	10/50 (20%)	19/50 (38%)
Adjusted rate	26.8%	13.4%	21.5%	42.4%
Terminal rate	9/32 (28%)	3/32 (9%)	8/38 (21%)	11/32 (34%)
First incidence (days)	562	576	632	558
Poly-3 test	P=0.014	P=0.091N	P=0.362N	P=0.089
All Organs: Hemangiosarcoma				
Overall rate	4/50 (8%)	4/50 (8%)	2/50 (4%)	5/50 (10%)
Adjusted rate	9.0%	8.9%	4.3%	11.4%
Terminal rate	2/32 (6%)	2/32 (6%)	1/38 (3%)	2/32 (6%)
First incidence (days)	578	542	637	429
Poly-3 test	P=0.441	P=0.638N	P=0.317N	P=0.491
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	5/50 (10%)	5/50 (10%)	3/50 (6%)	6/50 (12%)
Adjusted rate	11.2%	11.1%	6.5%	13.7%
Terminal rate	3/32 (9%)	3/32 (9%)	2/38 (5%)	3/32 (9%)
First incidence (days)	578	542	637	429
Poly-3 test	P=0.446	P=0.625N	P=0.332N	P=0.490
All Organs: Malignant Lymphoma				
Overall rate	4/50 (8%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate	9.1%	2.3%	4.3%	0.0%
Terminal rate	2/32 (6%)	1/32 (3%)	1/38 (3%)	0/32 (0%)
First incidence (days)	666	730 (T)	554	—
Poly-3 test	P=0.054N	P=0.178N	P=0.311N	P=0.066N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
All Organs: Benign Neoplasms				
Overall rate	38/50 (76%)	34/50 (68%)	40/50 (80%)	44/50 (88%)
Adjusted rate	78.2%	72.0%	81.5%	91.7%
Terminal rate	26/32 (81%)	24/32 (75%)	31/38 (82%)	29/32 (91%)
First incidence (days)	339	542	486	429
Poly-3 test	P=0.019	P=0.318N	P=0.438	P=0.050
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	22/50 (44%)	24/50 (48%)	22/50 (44%)
Adjusted rate	56.2%	47.2%	49.8%	48.5%
Terminal rate	17/32 (53%)	13/32 (41%)	17/38 (45%)	12/32 (38%)
First incidence (days)	562	542	554	429
Poly-3 test	P=0.322N	P=0.251N	P=0.339N	P=0.296N
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/50 (92%)	44/50 (88%)	46/50 (92%)	47/50 (94%)
Adjusted rate	93.6%	91.2%	92.0%	97.5%
Terminal rate	30/32 (94%)	29/32 (91%)	34/38 (90%)	31/32 (97%)
First incidence (days)	339	542	486	429
Poly-3 test	P=0.212	P=0.473N	P=0.532N	P=0.315

(T) Terminal sacrifice

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

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

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Ginseng[a]

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	12	10	8	8
Natural deaths	6	7	4	8
Survivors				
Died last week of study		1		1
Terminal sacrifice	32	32	38	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Arteriole, periesophageal tissue, inflammation		1 (2%)		
Periesophageal tissue, inflammation			1 (2%)	
Gallbladder	(49)	(50)	(49)	(50)
Cyst	1 (2%)		1 (2%)	
Inflammation				2 (4%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Serosa, inflammation	1 (2%)			
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	1 (2%)
Serosa, inflammation	1 (2%)			
Intestine small, jejunum	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Peyer's patch, hyperplasia				2 (4%)
Serosa, inflammation	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)			
Angiectasis	1 (2%)	1 (2%)		
Basophilic focus	3 (6%)	3 (6%)	4 (8%)	1 (2%)
Clear cell focus	11 (22%)	21 (42%)	21 (42%)	16 (32%)
Eosinophilic focus	17 (34%)	16 (32%)	18 (36%)	21 (42%)
Fibrosis	1 (2%)			
Hyperplasia, regenerative	1 (2%)			
Infarct				1 (2%)
Infiltration cellular, lymphocyte			1 (2%)	
Inflammation	8 (16%)	4 (8%)	7 (14%)	3 (6%)
Mixed cell focus	8 (16%)	6 (12%)	5 (10%)	5 (10%)
Tension lipidosis	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Thrombosis			1 (2%)	
Bile duct, inflammation				1 (2%)
Hepatocyte, atypia cellular	1 (2%)	1 (2%)		
Hepatocyte, necrosis	3 (6%)	4 (8%)	4 (8%)	1 (2%)
Hepatocyte, vacuolization, cytoplasmic, diffuse	12 (24%)	15 (30%)	11 (22%)	17 (34%)
Kupffer cell, pigmentation, hemosiderin		1 (2%)		
Mesentery	(3)	(4)	(6)	(0)
Necrosis	2 (67%)	2 (50%)	4 (67%)	
Artery, inflammation		1 (25%)		
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus	1 (2%)	1 (2%)	1 (2%)	
Infiltration cellular, lipocyte	1 (2%)			

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

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Alimentary System (continued)								
Salivary glands	(50)		(50)		(50)		(50)	
Infiltration cellular, mononuclear cell					1	(2%)		
Stomach, forestomach	(50)		(50)		(50)		(50)	
Erosion					1	(2%)		
Inflammation	1	(2%)			2	(4%)		
Ulcer	2	(4%)	1	(2%)	5	(10%)	1	(2%)
Epithelium, diverticulum	1	(2%)						
Epithelium, hyperplasia	5	(10%)	4	(8%)	3	(6%)	2	(4%)
Epithelium, hyperplasia, focal			1	(2%)				
Stomach, glandular	(50)		(50)		(50)		(50)	
Inflammation					1	(2%)		
Ulcer	1	(2%)			1	(2%)		
Epithelium, mineralization			1	(2%)	1	(2%)	1	(2%)
Tooth	(13)		(15)		(9)		(13)	
Dysplasia	11	(85%)	11	(73%)	8	(89%)	10	(77%)
Cardiovascular System								
Blood vessel	(50)		(48)		(50)		(50)	
Inflammation			1	(2%)				
Heart	(50)		(50)		(50)		(50)	
Cardiomyopathy	42	(84%)	43	(86%)	45	(90%)	41	(82%)
Hemorrhage			1	(2%)				
Inflammation			2	(4%)				
Mineralization	1	(2%)			1	(2%)		
Artery, inflammation			3	(6%)				
Artery, mineralization			1	(2%)				
Ventricle, thrombosis							1	(2%)
Endocrine System								
Adrenal cortex	(50)		(50)		(50)		(50)	
Hyperplasia	10	(20%)	16	(32%)	16	(32%)	6	(12%)
Hypertrophy	28	(56%)	24	(48%)	31	(62%)	35	(70%)
Subcapsular, hyperplasia, focal	1	(2%)						
Adrenal medulla	(50)		(50)		(50)		(50)	
Hyperplasia	2	(4%)			1	(2%)		
Islets, pancreatic	(50)		(50)		(50)		(50)	
Hyperplasia	38	(76%)	38	(76%)	38	(76%)	40	(80%)
Parathyroid gland	(49)		(48)		(45)		(48)	
Cyst					1	(2%)		
Pituitary gland	(50)		(50)		(50)		(50)	
Pars distalis, cyst	2	(4%)	1	(2%)	1	(2%)	5	(10%)
Pars distalis, hyperplasia	3	(6%)	4	(8%)	2	(4%)	3	(6%)
Pars intermedia, hyperplasia	1	(2%)						
Thyroid gland	(50)		(50)		(50)		(50)	
Follicle, cyst	1	(2%)			2	(4%)		
General Body System								
None								
Genital System								
Epididymis	(50)		(50)		(50)		(50)	
Granuloma sperm	2	(4%)	4	(8%)	1	(2%)	1	(2%)
Inflammation	2	(4%)	4	(8%)	6	(12%)	2	(4%)
Preputial gland	(50)		(50)		(50)		(50)	
Inflammation	5	(10%)	6	(12%)	4	(8%)	8	(16%)
Duct, ectasia	9	(18%)	7	(14%)	6	(12%)	12	(24%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Genital System (continued)								
Prostate	(50)		(50)		(50)		(50)	
Inflammation	3	(6%)			2	(4%)		
Epithelium, hyperplasia							1	(2%)
Seminal vesicle	(50)		(50)		(50)		(50)	
Dilatation					1	(2%)		
Inflammation							1	(2%)
Testes	(50)		(50)		(50)		(50)	
Mineralization	3	(6%)						
Germinal epithelium, atrophy	2	(4%)	4	(8%)	5	(10%)	4	(8%)
Interstitial cell, hyperplasia			1	(2%)				
Hematopoietic System								
Bone marrow	(50)		(50)		(50)		(50)	
Hyperplasia	1	(2%)			1	(2%)		
Lymph node	(2)		(2)		(2)		(1)	
Inguinal, hyperplasia, plasma cell							1	(100%)
Lymph node, mandibular	(50)		(50)		(50)		(50)	
Hyperplasia, plasma cell	1	(2%)						
Lymph node, mesenteric	(50)		(49)		(50)		(50)	
Hemorrhage			1	(2%)				
Hyperplasia, lymphoid	1	(2%)					1	(2%)
Hyperplasia, plasma cell	1	(2%)			1	(2%)		
Necrosis			1	(2%)				
Artery, inflammation			1	(2%)				
Spleen	(50)		(50)		(50)		(50)	
Hematopoietic cell proliferation	2	(4%)	5	(10%)	7	(14%)	6	(12%)
Hyperplasia, lymphoid	7	(14%)	11	(22%)	11	(22%)	4	(8%)
Lymphoid follicle, atrophy	5	(10%)	7	(14%)	1	(2%)	4	(8%)
Red pulp, atrophy	1	(2%)						
Vein, inflammation			1	(2%)				
Thymus	(47)		(50)		(50)		(49)	
Cyst					1	(2%)		
Inflammation			1	(2%)				
Arteriole, inflammation			1	(2%)				
Integumentary System								
Skin	(50)		(50)		(50)		(50)	
Inflammation			1	(2%)			2	(4%)
Ulcer	2	(4%)	1	(2%)	1	(2%)	2	(4%)
Subcutaneous tissue, edema			1	(2%)				
Musculoskeletal System								
Skeletal muscle	(1)		(1)		(0)		(0)	
Nervous System								
Brain	(50)		(50)		(50)		(50)	
Hemorrhage			1	(2%)				
Hippocampus, neuron, necrosis					1	(2%)		
Meninges, hemorrhage							1	(2%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Respiratory System								
Lung	(50)		(50)		(50)		(50)	
Hemorrhage	1	(2%)			2	(4%)		
Inflammation	4	(8%)	2	(4%)	2	(4%)	1	(2%)
Pigmentation, hemosiderin							1	(2%)
Thrombosis	1	(2%)			2	(4%)	1	(2%)
Alveolar epithelium, hyperplasia	3	(6%)	1	(2%)	6	(12%)		
Alveolus, infiltration cellular, histiocyte							1	(2%)
Arteriole, inflammation	1	(2%)						
Nose	(50)		(49)		(49)		(50)	
Inflammation	6	(12%)	5	(10%)	7	(14%)	6	(12%)
Polyp, inflammatory			3	(6%)				
Trachea	(50)		(50)		(50)		(50)	
Special Senses System								
Eye	(50)		(50)		(50)		(50)	
Cornea, inflammation	3	(6%)	3	(6%)	1	(2%)	3	(6%)
Optic nerve, atrophy	1	(2%)						
Retina, dysplasia	1	(2%)					1	(2%)
Retina, hemorrhage			1	(2%)				
Harderian gland	(50)		(50)		(50)		(50)	
Hyperplasia			2	(4%)	1	(2%)	1	(2%)
Inflammation					1	(2%)		
Urinary System								
Kidney	(50)		(50)		(50)		(50)	
Cyst	9	(18%)	6	(12%)	7	(14%)	7	(14%)
Hydronephrosis	2	(4%)	2	(4%)	3	(6%)	3	(6%)
Infarct	1	(2%)			1	(2%)	4	(8%)
Inflammation	2	(4%)	2	(4%)	1	(2%)	1	(2%)
Metaplasia, osseous					2	(4%)		
Mineralization					2	(4%)		
Nephropathy	48	(96%)	47	(94%)	48	(96%)	47	(94%)
Arteriole, inflammation			1	(2%)				
Artery, inflammation			2	(4%)	1	(2%)		
Papilla, necrosis	1	(2%)						
Pelvis, inflammation			1	(2%)			1	(2%)
Renal tubule, hyperplasia			2	(4%)	2	(4%)		
Vein, inflammation					1	(2%)		
Urinary bladder	(50)		(50)		(50)		(50)	
Inflammation	1	(2%)			1	(2%)		
Transitional epithelium, hyperplasia	1	(2%)						

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

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

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			1	4
Moribund	7	11	10	8
Natural deaths	5	8	5	6
Survivors				
Terminal sacrifice	38	31	34	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(49)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(49)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hemangioma		1 (2%)		
Hemangiosarcoma		1 (2%)	2 (4%)	
Hepatocellular adenoma	14 (28%)	10 (20%)	11 (22%)	6 (12%)
Hepatocellular adenoma, multiple	7 (14%)	10 (20%)	6 (12%)	9 (18%)
Hepatocellular carcinoma	2 (4%)	4 (8%)	4 (8%)	1 (2%)
Hepatocellular carcinoma, multiple		1 (2%)		
Osteosarcoma, metastatic, bone		1 (2%)		
Mesentery	(8)	(9)	(7)	(4)
Osteosarcoma, metastatic, bone		1 (11%)		
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Pericardium, osteosarcoma, metastatic, bone		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	1 (2%)			
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		1 (2%)	
Parathyroid gland	(41)	(44)	(39)	(43)
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, adenoma	1 (2%)	4 (8%)	5 (10%)	3 (6%)
Pars intermedia, adenoma	1 (2%)	1 (2%)		
Thyroid gland	(49)	(50)	(50)	(50)
C-cell, carcinoma			1 (2%)	
Follicular cell, carcinoma			1 (2%)	
General Body System				
None				

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Genital System								
Clitoral gland	(50)		(50)		(49)		(50)	
Ovary	(50)		(50)		(50)		(50)	
Cystadenoma	7	(14%)	2	(4%)	3	(6%)		
Hemangioma					1	(2%)		
Luteoma					1	(2%)		
Periovarian tissue, fibrous histiocytoma, metastatic, skin	1	(2%)						
Uterus	(50)		(50)		(50)		(50)	
Carcinoma							1	(2%)
Hemangioma					1	(2%)		
Hemangiosarcoma					1	(2%)		
Polyp stromal					2	(4%)		
Sarcoma stromal							1	(2%)
Hematopoietic System								
Bone marrow	(50)		(50)		(49)		(50)	
Hemangiosarcoma, metastatic, spleen							1	(2%)
Lymph node	(4)		(4)		(6)		(6)	
Fibrosarcoma, metastatic, skin	1	(25%)						
Lymph node, mandibular	(50)		(49)		(50)		(50)	
Lymph node, mesenteric	(49)		(50)		(50)		(50)	
Spleen	(50)		(50)		(49)		(50)	
Hemangioma	1	(2%)						
Hemangiosarcoma							1	(2%)
Thymus	(48)		(49)		(49)		(49)	
Osteosarcoma, metastatic, bone			1	(2%)				
Integumentary System								
Mammary gland	(50)		(50)		(50)		(50)	
Adenoma	1	(2%)						
Skin	(50)		(50)		(50)		(50)	
Sarcoma			1	(2%)				
Trichoepithelioma							1	(2%)
Subcutaneous tissue, fibrosarcoma	3	(6%)					2	(4%)
Subcutaneous tissue, fibrous histiocytoma	1	(2%)					3	(6%)
Subcutaneous tissue, hemangioma							1	(2%)
Subcutaneous tissue, hemangiosarcoma			1	(2%)				
Subcutaneous tissue, sarcoma			2	(4%)	1	(2%)		
Subcutaneous tissue, schwannoma, malignant	2	(4%)			1	(2%)	2	(4%)
Musculoskeletal System								
Bone	(50)		(50)		(50)		(50)	
Osteosarcoma			2	(4%)				
Nervous System								
Brain	(50)		(50)		(50)		(50)	
Spinal cord	(0)		(1)		(1)		(1)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Respiratory System								
Lung	(50)		(50)		(50)		(50)	
Alveolar/bronchiolar adenoma	5	(10%)	2	(4%)	2	(4%)	4	(8%)
Alveolar/bronchiolar adenoma, multiple	1	(2%)	1	(2%)				
Alveolar/bronchiolar carcinoma	1	(2%)	2	(4%)				
Alveolar/bronchiolar carcinoma, multiple			1	(2%)	1	(2%)		
Carcinoma, metastatic, thyroid gland					1	(2%)		
Fibrous histiocytoma, metastatic, skin	1	(2%)						
Hepatocellular carcinoma, metastatic, liver	1	(2%)	3	(6%)	2	(4%)		
Osteosarcoma, metastatic, bone			2	(4%)				
Osteosarcoma, metastatic, uncertain primary site					1	(2%)		
Sarcoma, metastatic, skin			1	(2%)				
Nose	(50)		(50)		(50)		(49)	
Adenoma			1	(2%)				
Special Senses System								
Eye	(49)		(50)		(50)		(50)	
Harderian gland	(49)		(50)		(50)		(50)	
Adenoma	5	(10%)	7	(14%)	8	(16%)	7	(14%)
Bilateral, adenoma	2	(4%)			1	(2%)	1	(2%)
Urinary System								
Kidney	(50)		(50)		(50)		(50)	
Urinary bladder	(49)		(50)		(50)		(50)	
Systemic Lesions								
Multiple organs[b]	(50)		(50)		(50)		(50)	
Histiocytic sarcoma			1	(2%)	1	(2%)	2	(4%)
Lymphoma malignant	9	(18%)	12	(24%)	6	(12%)	8	(16%)
Neoplasm Summary								
Total animals with primary neoplasms[c]	42		38		34		36	
Total primary neoplasms	65		67		62		53	
Total animals with benign neoplasms	33		30		29		25	
Total benign neoplasms	47		39		43		32	
Total animals with malignant neoplasms	15		27		16		20	
Total malignant neoplasms	18		28		19		21	
Total animals with metastatic neoplasms	3		6		4		1	
Total metastatic neoplasms	4		10		4		1	
Total animals with malignant neoplasms of uncertain primary site					1			

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

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	7/50 (14%)	7/50 (14%)	9/50 (18%)	8/50 (16%)
Adjusted rat[b]	14.9%	16.0%	20.6%	19.6%
Terminal rate[c]	5/38 (13%)	6/31 (19%)	6/34 (18%)	8/32 (25%)
First incidence (days)	697	618	654	729 (T)
Poly-3 test[d]	P=0.290	P=0.558	P=0.332	P=0.381
Liver: Hepatocellular Adenoma				
Overall rate	21/50 (42%)	20/50 (40%)	17/50 (34%)	15/50 (30%)
Adjusted rate	44.1%	45.6%	39.3%	35.9%
Terminal rate	18/38 (47%)	17/31 (55%)	16/34 (47%)	12/32 (38%)
First incidence (days)	578	648	715	555
Poly-3 test	P=0.204N	P=0.527	P=0.403N	P=0.285N
Liver: Hepatocellular Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	4/50 (8%)	1/50 (2%)
Adjusted rate	4.3%	11.4%	9.2%	2.4%
Terminal rate	2/38 (5%)	2/31 (7%)	2/34 (6%)	0/32 (0%)
First incidence (days)	729 (T)	633	711	677
Poly-3 test	P=0.352N	P=0.190	P=0.301	P=0.547N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	23/50 (46%)	21/50 (42%)	19/50 (38%)	16/50 (32%)
Adjusted rate	48.3%	47.5%	43.9%	38.1%
Terminal rate	20/38 (53%)	17/31 (55%)	17/34 (50%)	12/32 (38%)
First incidence (days)	578	633	711	555
Poly-3 test	P=0.172N	P=0.553N	P=0.417N	P=0.225N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/50 (12%)	3/50 (6%)	2/50 (4%)	4/50 (8%)
Adjusted rate	12.8%	6.9%	4.6%	9.8%
Terminal rate	6/38 (16%)	3/31 (10%)	2/34 (6%)	4/32 (13%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.384N	P=0.281N	P=0.161N	P=0.460N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.1%	6.8%	2.3%	0.0%
Terminal rate	1/38 (3%)	2/31 (7%)	0/34 (0%)	0/32 (0%)
First incidence (days)	729 (T)	550	725	—[e]
Poly-3 test	P=0.242N	P=0.283	P=0.742	P=0.528N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	7/50 (14%)	6/50 (12%)	3/50 (6%)	4/50 (8%)
Adjusted rate	14.9%	13.6%	7.0%	9.8%
Terminal rate	7/38 (18%)	5/31 (16%)	2/34 (6%)	4/32 (13%)
First incidence (days)	729 (T)	550	725	729 (T)
Poly-3 test	P=0.221N	P=0.548N	P=0.193N	P=0.346N
Ovary: Cystadenoma				
Overall rate	7/50 (14%)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	14.9%	4.6%	7.0%	0.0%
Terminal rate	7/38 (18%)	2/31 (7%)	3/34 (9%)	0/32 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	—
Poly-3 test	P=0.010N	P=0.098N	P=0.193N	P=0.013N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	1/50 (2%)	4/50 (8%)	5/50 (10%)	3/49 (6%)
Adjusted rate	2.1%	9.2%	11.3%	7.5%
Terminal rate	1/38 (3%)	2/31 (7%)	3/34 (9%)	2/31 (7%)
First incidence (days)	729 (T)	704	444	719
Poly-3 test	P=0.232	P=0.157	P=0.088	P=0.250
Skin (Subcutaneous): Fibrous Histiocytoma				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.1%	0.0%	0.0%	7.2%
Terminal rate	0/38 (0%)	0/31 (0%)	0/34 (0%)	0/32 (0%)
First incidence (days)	673	—	—	644
Poly-3 test	P=0.080	P=0.516N	P=0.517N	P=0.261
Skin (Subcutaneous): Fibrosarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adjusted rate	6.3%	0.0%	0.0%	4.9%
Terminal rate	1/38 (3%)	0/31 (0%)	0/34 (0%)	2/32 (6%)
First incidence (days)	590	—	—	729 (T)
Poly-3 test	P=0.524N	P=0.135N	P=0.137N	P=0.568N
Skin (Subcutaneous): Sarcoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	6.9%	2.3%	0.0%
Terminal rate	0/38 (0%)	2/31 (7%)	0/34 (0%)	0/32 (0%)
First incidence (days)	—	648	673	—
Poly-3 test	P=0.427N	P=0.107	P=0.484	—[f]
Skin (Subcutaneous): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	4/50 (8%)	3/50 (6%)	1/50 (2%)	5/50 (10%)
Adjusted rate	8.4%	6.9%	2.3%	12.1%
Terminal rate	1/38 (3%)	2/31 (7%)	0/34 (0%)	2/32 (6%)
First incidence (days)	590	648	673	644
Poly-3 test	P=0.356	P=0.546N	P=0.209N	P=0.414
All Organs: Hemangiosarcoma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	4.5%	7.0%	2.5%
Terminal rate	0/38 (0%)	1/31 (3%)	3/34 (9%)	1/32 (3%)
First incidence (days)	—	428	729 (T)	729 (T)
Poly-3 test	P=0.360	P=0.225	P=0.105	P=0.472
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	5/50 (10%)	2/50 (4%)
Adjusted rate	2.1%	6.8%	11.6%	4.9%
Terminal rate	1/38 (3%)	1/31 (3%)	5/34 (15%)	2/32 (6%)
First incidence (days)	729 (T)	428	729 (T)	729 (T)
Poly-3 test	P=0.340	P=0.285	P=0.083	P=0.452
All Organs: Malignant Lymphoma				
Overall rate	9/50 (18%)	12/50 (24%)	6/50 (12%)	8/50 (16%)
Adjusted rate	18.9%	26.6%	13.9%	19.0%
Terminal rate	4/38 (11%)	8/31 (26%)	6/34 (18%)	4/32 (13%)
First incidence (days)	677	508	729 (T)	493
Poly-3 test	P=0.401N	P=0.263	P=0.360N	P=0.601

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
All Organs: Benign Neoplasms				
Overall rate	33/50 (66%)	30/50 (60%)	29/50 (58%)	25/50 (50%)
Adjusted rate	69.0%	66.7%	65.1%	59.8%
Terminal rate	28/38 (74%)	23/31 (74%)	24/34 (71%)	21/32 (66%)
First incidence (days)	578	550	444	555
Poly-3 test	P=0.198N	P=0.493N	P=0.427N	P=0.241N
All Organs: Malignant Neoplasms				
Overall rate	15/50 (30%)	27/50 (54%)	17/50 (34%)	20/50 (40%)
Adjusted rate	30.8%	56.0%	38.2%	44.9%
Terminal rate	6/38 (16%)	15/31 (48%)	10/34 (29%)	9/32 (28%)
First incidence (days)	590	428	521	410
Poly-3 test	P=0.258	P=0.009	P=0.296	P=0.115
All Organs: Benign or Malignant Neoplasms				
Overall rate	42/50 (84%)	38/50 (76%)	35/50 (70%)	36/50 (72%)
Adjusted rate	85.0%	78.0.%	76.8%	79.8%
Terminal rate	31/38 (82%)	24/31 (77%)	26/34 (77%)	24/32 (75%)
First incidence (days)	578	428	444	410
Poly-3 test	P=0.336N	P=0.257N	P=0.222N	P=0.344N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. 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 could not be computed

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Ginseng[a]

	Vehicle Control	1,250 mg/kg	2,500 mg/kg	5,000 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			1	4
Moribund	7	11	10	8
Natural deaths	5	8	5	6
Survivors				
Terminal sacrifice	38	31	34	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Perforation			2 (4%)	
Muscularis, degeneration	1 (2%)			
Periesophageal tissue, inflammation			3 (6%)	1 (2%)
Gallbladder	(49)	(50)	(50)	(50)
Cyst		2 (4%)	1 (2%)	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	(49)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)		2 (4%)	
Basophilic focus	1 (2%)			2 (4%)
Clear cell focus	4 (8%)	3 (6%)	2 (4%)	2 (4%)
Eosinophilic focus	15 (30%)	14 (28%)	3 (6%)	9 (18%)
Hyperplasia, regenerative		1 (2%)		
Infiltration cellular, lymphocyte			1 (2%)	
Inflammation	14 (28%)	11 (22%)	10 (20%)	14 (28%)
Mixed cell focus	2 (4%)	5 (10%)	2 (4%)	2 (4%)
Tension lipidosis	2 (4%)	5 (10%)	4 (8%)	8 (16%)
Thrombosis			1 (2%)	1 (2%)
Hepatocyte, atypia cellular		1 (2%)		
Hepatocyte, necrosis	4 (8%)	2 (4%)		1 (2%)
Hepatocyte, vacuolization cytoplasmic, diffuse	8 (16%)	10 (20%)	10 (20%)	5 (10%)
Mesentery	(8)	(9)	(7)	(4)
Inflammation			1 (14%)	
Necrosis	8 (100%)	8 (89%)	5 (71%)	4 (100%)
Fat, necrosis			1 (14%)	
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus		2 (4%)		
Hemorrhage		1 (2%)		
Infiltration cellular, lipocyte		3 (6%)		
Inflammation		2 (4%)		
Acinus, hyperplasia	4 (8%)	1 (2%)	1 (2%)	
Artery, inflammation			1 (2%)	
Duct, cyst	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Infiltration cellular, mast cell			1 (2%)	
Inflammation		1 (2%)	1 (2%)	
Ulcer			1 (2%)	
Epithelium, hyperplasia	2 (4%)	2 (4%)	3 (6%)	4 (8%)
Epithelium, hyperplasia, focal		1 (2%)	2 (4%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion		1 (2%)	1 (2%)	
Ulcer			1 (2%)	
Epithelium, mineralization	1 (2%)	1 (2%)		

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

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Cardiovascular System								
Blood vessel	(50)		(50)		(50)		(50)	
Inflammation	1	(2%)			1	(2%)	1	(2%)
Mineralization			1	(2%)				
Media, hyperplasia	1	(2%)						
Heart	(50)		(50)		(50)		(50)	
Cardiomyopathy	35	(70%)	40	(80%)	38	(76%)	31	(62%)
Inflammation			2	(4%)	3	(6%)	1	(2%)
Mineralization			2	(4%)				
Artery, inflammation							2	(4%)
Atrium, thrombosis			1	(2%)				
Epicardium, hyperplasia			1	(2%)				
Myocardium, hyperplasia, reticulum cell	1	(2%)						
Valve, thrombosis			2	(4%)				
Ventricle, thrombosis	1	(2%)					1	(2%)
Endocrine System								
Adrenal cortex	(50)		(50)		(50)		(50)	
Hyperplasia	8	(16%)	1	(2%)	3	(6%)	6	(12%)
Hypertrophy	39	(78%)	40	(80%)	39	(78%)	39	(78%)
Adrenal medulla	(50)		(50)		(50)		(50)	
Amyloid deposition			1	(2%)				
Hyperplasia	3	(6%)	3	(6%)	6	(12%)	2	(4%)
Islets, pancreatic	(50)		(50)		(50)		(50)	
Hyperplasia	16	(32%)	15	(30%)	17	(34%)	15	(30%)
Parathyroid gland	(41)		(44)		(39)		(43)	
Hyperplasia							1	(2%)
Pituitary gland	(50)		(50)		(50)		(49)	
Pars distalis, cyst			2	(4%)				
Pars distalis, hyperplasia	18	(36%)	18	(36%)	19	(38%)	18	(37%)
Thyroid gland	(49)		(50)		(50)		(50)	
Inflammation							1	(2%)
C-cell, hyperplasia					1	(2%)		
Follicle, cyst			1	(2%)	2	(4%)	1	(2%)
General Body System								
None								
Genital System								
Clitoral gland	(50)		(50)		(49)		(50)	
Inflammation							1	(2%)
Ovary	(50)		(50)		(50)		(50)	
Cyst	4	(8%)	7	(14%)	6	(12%)	2	(4%)
Hemorrhage			1	(2%)	2	(4%)	1	(2%)
Inflammation	2	(4%)			2	(4%)		
Thrombosis	1	(2%)						
Uterus	(50)		(50)		(50)		(50)	
Angiectasis			1	(2%)			1	(2%)
Atrophy			1	(2%)				
Hemorrhage	1	(2%)					1	(2%)
Hydrometra					1	(2%)		
Inflammation	2	(4%)	2	(4%)	2	(4%)		
Thrombosis					1	(2%)		
Endometrium, decidual reaction					1	(2%)		
Endometrium, hyperplasia, cystic	47	(94%)	40	(80%)	35	(70%)	43	(86%)
Myometrium, atypia cellular	1	(2%)						

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Hematopoietic System								
Bone marrow	(50)		(50)		(49)		(50)	
Angiectasis	1	(2%)						
Hyperplasia					1	(2%)	2	(4%)
Lymph node	(4)		(4)		(6)		(6)	
Hyperplasia					1	(17%)		
Iliac, hemorrhage					1	(17%)		
Lumbar, hyperplasia, plasma cell	1	(25%)						
Renal, degeneration, cystic							1	(17%)
Renal, hemorrhage			2	(50%)	1	(17%)		
Lymph node, mandibular	(50)		(49)		(50)		(50)	
Amyloid deposition							1	(2%)
Hyperplasia					1	(2%)	1	(2%)
Hyperplasia, lymphoid					1	(2%)		
Infiltration cellular, histiocyte					2	(4%)		
Lymph node, mesenteric	(49)		(50)		(50)		(50)	
Degeneration, cystic			1	(2%)				
Hemorrhage			1	(2%)				
Hyperplasia, lymphoid			1	(2%)				
Infiltration cellular, histiocyte					2	(4%)		
Inflammation					2	(4%)	1	(2%)
Spleen	(50)		(50)		(49)		(50)	
Amyloid deposition					1	(2%)		
Atrophy							1	(2%)
Hematopoietic cell proliferation	4	(8%)	2	(4%)	2	(4%)	2	(4%)
Hyperplasia, lymphoid	22	(44%)	23	(46%)	24	(49%)	12	(24%)
Hyperplasia, plasma cell					1	(2%)		
Infarct	1	(2%)						
Lymphoid follicle, atrophy	2	(4%)	1	(2%)	4	(8%)	3	(6%)
Thymus	(48)		(49)		(49)		(49)	
Cyst	1	(2%)						
Hyperplasia, lymphoid							1	(2%)
Infiltration cellular, mast cell					1	(2%)		
Inflammation					1	(2%)		
Integumentary System								
Mammary gland	(50)		(50)		(50)		(50)	
Hyperplasia, focal			2	(4%)	3	(6%)	2	(4%)
Inflammation	1	(2%)						
Skin	(50)		(50)		(50)		(50)	
Inflammation	1	(2%)			1	(2%)		
Ulcer	1	(2%)	1	(2%)				
Subcutaneous tissue, fibrosis	2	(4%)	4	(8%)	1	(2%)	1	(2%)
Musculoskeletal System								
Bone	(50)		(50)		(50)		(50)	
Femur, fibro-osseous lesion			4	(8%)	2	(4%)	4	(8%)
Nervous System								
Brain	(50)		(50)		(50)		(50)	
Hydrocephalus			2	(4%)	1	(2%)		
Inflammation			1	(2%)				
Artery, inflammation					1	(2%)		
Meninges, inflammation					1	(2%)		
Spinal cord	(0)		(1)		(1)		(1)	
Degeneration							1	(100%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Ginseng

	Vehicle Control		1,250 mg/kg		2,500 mg/kg		5,000 mg/kg	
Respiratory System								
Lung	(50)		(50)		(50)		(50)	
Congestion							3	(6%)
Hemorrhage			1	(2%)	1	(2%)		
Inflammation	2	(4%)	6	(12%)	2	(4%)	6	(12%)
Thrombosis					2	(4%)		
Alveolar epithelium, hyperplasia	2	(4%)	3	(6%)	2	(4%)	1	(2%)
Alveolus, infiltration cellular, histiocyte					2	(4%)		
Nose	(50)		(50)		(50)		(49)	
Inflammation	1	(2%)			4	(8%)	3	(6%)
Special Senses System								
Eye	(49)		(50)		(50)		(50)	
Developmental malformation	1	(2%)						
Cornea, inflammation	2	(4%)	1	(2%)	3	(6%)		
Optic nerve, degeneration							1	(2%)
Harderian gland	(49)		(50)		(50)		(50)	
Hyperplasia	2	(4%)	1	(2%)	1	(2%)		
Urinary System								
Kidney	(50)		(50)		(50)		(50)	
Cyst			1	(2%)				
Infarct			2	(4%)			6	(12%)
Inflammation	7	(14%)	2	(4%)			1	(2%)
Metaplasia, osseous			3	(6%)	2	(4%)		
Mineralization			1	(2%)				
Nephropathy	39	(78%)	39	(78%)	38	(76%)	33	(66%)
Arteriole, inflammation							1	(2%)
Artery, inflammation			1	(2%)	1	(2%)		
Urinary bladder	(49)		(50)		(50)		(50)	
Inflammation							1	(2%)

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Testing performed by BioReliance Corporation (Rockville, MD) followed protocols reported by Zeiger *et al.* (1992); in the test conducted at SITEK Research Laboratories (Rockville, MD), a slightly modified procedure was used as described below. Ginseng was sent to both laboratories as a coded sample. The study conducted at SITEK Research Laboratories used the same lot of ginseng used for the 2-year study (lot 3031978). In tests conducted at BioReliance Corporation, ginseng was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA102, TA104, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following 2 days incubation at 37° C. The modified protocol used at SITEK Research Laboratories used only 10% rat liver S9 for exogenous metabolic activation and employed *Escherichia coli* strain WP2 *uvrA/*pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. Incubation of bacterial strains with ginseng and subsequent plating were carried out as described above for the traditional protocol.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of ginseng. For the study conducted at BioReliance Corporation, the high dose was limited by toxicity and precipitation to 3,333 µg/plate; at SITEK Research Laboratories, the assay limit dose used was 10,000 µg/plate. All trials were repeated, and those conducted with S9 activation enzymes were repeated using either the same or higher concentrations of S9.

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

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al*. (1990). At the termination of the 3-month toxicity study of ginseng, peripheral blood samples were obtained from male and female B6C3F1 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 or mature erythrocytes) in each of five animals per dose group. In addition, the percentage of polychromatic erythrocytes (PCEs or reticulocytes) in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dosed groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the vehicle control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed, and the magnitudes of those effects.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

Ginseng (with doses up to 3,333 µg/plate in the first study and 10,000 µg/plate in the second study) was not mutagenic in either of two independent bacterial mutagenicity assays, with or without exogenous metabolic activation (Table E1; Zeiger *et al*., 1992). Bacterial strains tested in the first study included *S. typhimurium* strains TA97, TA98, TA100, TA102, TA104, and TA1535, with and without 10% or 30% hamster or rat liver S9. Strains tested in the second study included *S. typhimurium* strains TA98 and TA100, and *E. coli* strain WP2 *uvrA/*pKM101, with and without 10% rat liver S9. A precipitate was noted in the first study at doses of 1,000 and 3,333 µg/plate; however, this did not interfere with colony growth or enumeration. In addition to the negative results in the two bacterial tests, no significant increases in the frequencies of micronucleated NCEs were seen in male or female B6C3F1 mice administered 1,000 to 5,000 mg/kg ginseng for 3 months by gavage (Table E2). The percentage of PCEs in the peripheral blood of male and female mice was unaltered by chemical exposure, suggesting a lack of ginseng-associated bone marrow toxicity.

TABLE E1
Mutagenicity of Ginseng in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
Study performed at BioReliance Corporation								
TA102	0	362 ± 13	288 ± 6	306 ± 5	289 ± 5	270 ± 37	324 ± 19	400 ± 39
	33	335 ± 18	292 ± 8	295 ± 6	295 ± 23	368 ± 21	275 ± 4	377 ± 18
	100	302 ± 8	311 ± 8	303 ± 15	304 ± 12	405 ± 5	303 ± 9	371 ± 11
	333	347 ± 38	314 ± 10	302 ± 15	310 ± 14	414 ± 23	280 ± 12	380 ± 4
	1,000	269 ± 9[c]	259 ± 31	279 ± 6[c]	287 ± 10[c]	403 ± 19[c]	302 ± 10[c]	420 ± 17[c]
	3,333	302 ± 9[c]	218 ± 20[c]	290 ± 15[c]	253 ± 16[c]	411 ± 20[c]	306 ± 12[c]	409 ± 21[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		1,742 ± 61	971 ± 27	1,055 ± 30	1,873 ± 25	1,723 ± 61	2,081 ± 33	1,590 ± 35
TA104	0	249 ± 13	251 ± 5		359 ± 9	326 ± 11	304 ± 3	284 ± 16
	33	268 ± 3	268 ± 2		327 ± 11	319 ± 5	317 ± 3	254 ± 19
	100	236 ± 19	255 ± 4[e]		336 ± 25	340 ± 2	307 ± 6	282 ± 8
	333	249 ± 9	295 ± 22		276 ± 15	364 ± 1	314 ± 4	297 ± 8
	1,000	220 ± 8[c]	248 ± 14[c]		375 ± 7[c]	333 ± 9[c]	357 ± 27[c]	263 ± 27[c]
	3,333	221 ± 15[c]	274 ± 29[c]		295 ± 25[c]	344 ± 20[c]	297 ± 9[c]	309 ± 8[c]
Trial summary		Negative	Negative		Negative	Negative	Negative	Negative
Positive control		756 ± 13	796 ± 10		1,736 ± 65	1,494 ± 45	2,131 ± 20	897 ± 87
TA100	0	101 ± 7	97 ± 2		89 ± 1	84 ± 13[e]	95 ± 4	81 ± 3
	33	102 ± 2	95 ± 3		88 ± 1	78 ± 3	88 ± 5	84 ± 4
	100	106 ± 2	93 ± 3		92 ± 2	97 ± 0	97 ± 3	71 ± 2
	333	104 ± 5	97 ± 1		98 ± 3	79 ± 4	87 ± 5	89 ± 4
	1,000	97 ± 3[c]	88 ± 3[c]		98 ± 8[c]	68 ± 2[c]	97 ± 6[e]	75 ± 2[c]
	3,333	88 ± 4[f]	92 ± 2[c]		115 ± 10[c]	67 ± 10[c]	103 ± 6[c]	67 ± 3[f]
Trial summary		Negative	Negative		Negative	Negative	Negative	Negative
Positive control		211 ± 4	285 ± 10		483 ± 77	236 ± 15	456 ± 21	341 ± 16
TA1535	0	16 ± 0	8 ± 1		10 ± 1	14 ± 1	12 ± 1	13 ± 3
	33	19 ± 3	10 ± 2[e]		10 ± 2	14 ± 1	12 ± 3	14 ± 1
	100	15 ± 3	11 ± 3		10 ± 2	11 ± 1	9 ± 2	12 ± 1
	333	15 ± 3	8 ± 0		9 ± 2	16 ± 3	14 ± 2	11 ± 1
	1,000	13 ± 0	9 ± 2[c]		9 ± 1[c]	11 ± 1	8 ± 0	11 ± 1
	3,333	12 ± 1[c]	8 ± 2[c]		7 ± 1[c]	9 ± 2[c]	13 ± 2[c]	16 ± 4[c]
Trial summary		Negative	Negative		Negative	Negative	Negative	Negative
Positive control		204 ± 9	175 ± 9		66 ± 2	212 ± 17	138 ± 18	107 ± 24
TA97	0	121 ± 14	94 ± 4		116 ± 2	148 ± 9	111 ± 5	199 ± 20
	33	131 ± 2	97 ± 8		125 ± 8	159 ± 9	117 ± 5	173 ± 14
	100	123 ± 6	101 ± 2		108 ± 4	161 ± 16	123 ± 5	185 ± 4
	333	130 ± 15	97 ± 6		116 ± 13	163 ± 9	122 ± 5	198 ± 14
	1,000	127 ± 7	86 ± 4[c]		113 ± 10[c]	138 ± 9	121 ± 3	160 ± 16
	3,333	124 ± 7[f]	83 ± 1[c]		89 ± 8[c]	155 ± 9[c]	97 ± 2[c]	156 ± 2[c]
Trial Summary		Negative	Negative		Negative	Negative	Negative	Negative
Positive Control		246 ± 22	357 ± 50[e]		691 ± 196	858 ± 74	1,379 ± 130	613 ± 37
TA98	0	25 ± 2	12 ± 1		13 ± 1	17 ± 1	14 ± 1	22 ± 2
	33	23 ± 1	11 ± 2		14 ± 1	20 ± 2	12 ± 1	21 ± 1
	100	22 ± 4	12 ± 0		12 ± 1	20 ± 3	10 ± 1	23 ± 1
	333	22 ± 3	13 ± 2		13 ± 2	21 ± 1	14 ± 2	25 ± 1
	1,000	17 ± 2[c]	15 ± 2[c]		18 ± 1[c]	17 ± 2[c]	11 ± 2	17 ± 1[c]
	3,333	17 ± 3[c]	12 ± 2[c]		17 ± 2[c]	15 ± 2[c]	15 ± 0[c]	18 ± 3[c]
Trial summary		Negative	Negative		Negative	Negative	Negative	Negative
Positive control		65 ± 4	121 ± 14		465 ± 90	71 ± 7	189 ± 12	84 ± 12

TABLE E1
Mutagenicity of Ginseng in Bacterial Tester Strains

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+ 10% rat S9 Trial 1	+ 10% rat S9 Trial 2	+ 10% rat S9 Trial 3
Study performed at SITEK Research Laboratories (Lot 3031978 used in the 2-year gavage studies)							
TA100	0	58 ± 6	87 ± 3		67 ± 6	102 ± 2	
	1,500	69 ± 7	89 ± 5		74 ± 5	104 ± 3	
	2,500	54 ± 3	95 ± 2		93 ± 9	105 ± 4	
	5,000	70 ± 3	99 ± 6		81 ± 4	104 ± 5	
	7,500	63 ± 2	100 ± 3		88 ± 3	104 ± 6	
	10,000	85 ± 7	100 ± 7		120 ± 25	93 ± 9	
		Negative	Negative		Equivocal	Negative	
		413 ± 39	464 ± 12		1,010 ± 66	925 ± 40	
TA98	0	21 ± 1	19 ± 2	25 ± 2	35 ± 3	29 ± 2	28 ± 3
	1,500	20 ± 3	62 ± 6	23 ± 2	35 ± 5	33 ± 1	31 ± 4
	2,500	26 ± 4	56 ± 4	25 ± 2	35 ± 5	35 ± 4	31 ± 3
	5,000	29 ± 4	60 ± 2	28 ± 2	39 ± 1	30 ± 2	36 ± 1
	7,500	30 ± 5	69 ± 5	31 ± 4	37 ± 4	35 ± 1	39 ± 4
	10,000	22 ± 6	92 ± 2	36 ± 2	29 ± 2	38 ± 2	34 ± 2
Trial summary		Negative	Positive	Negative	Negative	Negative	Negative
Positive control		412 ± 6	464 ± 12	487 ± 31	757 ± 40	560 ± 24	1,137 ± 42
***Escherichia coli* WP2 *uvrA*/pKM101 (Analogous to *Salmonella typhimurium* TA102)**							
	0	225 ± 14	194 ± 9		242 ±2	229 ± 10	
	1,500	234 ± 8	221 ± 1		241 ± 5	247 ± 11	
	2,500	230 ± 8	271 ± 25		245 ± 7	266 ± 76	
	5,000	153 ± 5	189 ± 9		233 ± 6	212 ± 31	
	7,500	131 ± 2	216 ± 12		237 ± 13	259 ± 9	
	10,000	127 ± 5	56 ± 7		236 ± 7	212 ± 33	
Trial summary		Negative	Negative		Negative	Negative	
Positive control		2,016 ± 73	1,195 ± 41		1,077 ± 58	1,073 ± 7	

[a] The detailed protocol used by BioReliance Corporation and these data are presented by Zeiger *et al*. (1992); SITEK Research Laboratories used a modification of this protocol. 0 µg/plate is the solvent control

[b] Revertants are presented as mean ± standard error from three plates

[c] Precipitate on plate

[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), 4-nitro-*o*-phenylenediamine (TA98), mitomycin-C (TA102), and methyl methanesulfonate (TA104 and *E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene, and 2-aminoanthracene or sterigmatocystin was used for TA102.

[e] Contamination

[f] Slight toxicity and precipitate on plate

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Ginseng by Gavage for 3 Months[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Sterile water[d]	0	5	2.80 ± 0.20		3.84 ± 0.29
Ginseng	1,000	5	2.40 ± 0.19	0.7107	3.76 ± 0.33
	2,000	5	2.60 ± 0.48	0.6074	4.62 ± 0.63
	3,000	5	1.90 ± 0.48	0.9056	3.86 ± 0.26
	4,000	5	2.50 ± 0.42	0.6601	3.40 ± 0.19
	5,000	5	3.40 ± 0.19	0.2227	3.56 ± 0.20
			P=0.271[e]		
Female					
Sterile water	0	5	1.70 ± 0.20		3.78 ± 0.36
Ginseng	1,000	5	1.70 ± 0.20	0.5000	4.26 ± 0.46
	2,000	5	1.60 ± 0.29	0.5692	4.16 ± 0.11
	3,000	5	2.00 ± 0.39	0.3108	3.98 ± 0.32
	4,000	5	2.10 ± 0.40	0.2580	4.00 ± 0.24
	5,000	5	1.50 ± 0.16	0.6383	4.26 ± 0.25
			P=0.432		

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

APPENDIX F
CLINICAL PATHOLOGY RESULTS

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

	Vehicle Control	1,000 mg/kg	2,000 mg/kg	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	10	10	10
Day 23	9	10	9	10	10	8
Week 14	10	10	10	10	9	10
Hematocrit (%)						
Day 4	45.1 ± 0.5	44.9 ± 0.5	46.0 ± 0.8	45.7 ± 0.6	45.5 ± 1.1	44.3 ± 1.0
Day 23	48.8 ± 0.6	48.6 ± 0.4	47.4 ± 0.8	47.9 ± 0.5	48.0 ± 0.5	48.8 ± 0.7
Week 14	47.9 ± 0.5	47.6 ± 0.5	46.8 ± 0.3	47.8 ± 0.5	49.0 ± 0.4	48.8 ± 0.8
Hemoglobin (g/dL)						
Day 4	13.2 ± 0.2	13.2 ± 0.1	13.6 ± 0.2	13.5 ± 0.2	13.4 ± 0.3	13.0 ± 0.3
Day 23	14.9 ± 0.2	14.8 ± 0.1	14.6 ± 0.2	14.5 ± 0.1	14.8 ± 0.1	15.1 ± 0.2
Week 14	15.3 ± 0.2	15.3 ± 0.1	15.1 ± 0.1	15.2 ± 0.2	15.5 ± 0.1	15.5 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	7.21 ± 0.08	7.18 ± 0.08	7.30 ± 0.13	7.27 ± 0.09	7.14 ± 0.15	7.06 ± 0.16
Day 23	8.04 ± 0.08	8.04 ± 0.09	7.84 ± 0.09	7.89 ± 0.09	7.85 ± 0.10	8.04 ± 0.09
Week 14	8.98 ± 0.07	8.91 ± 0.10	8.79 ± 0.04	8.95 ± 0.10	9.23 ± 0.09	9.18 ± 0.17
Mean cell volume (fL)						
Day 4	62.5 ± 0.3	62.5 ± 0.3	63.0 ± 0.3	62.8 ± 0.2	63.7 ± 0.3*	62.8 ± 0.3
Day 23	60.6 ± 0.3	60.4 ± 0.2	60.5 ± 0.4	60.7 ± 0.2	61.1 ± 0.3	60.6 ± 0.4
Week 14	53.3 ± 0.3	53.4 ± 0.4	53.2 ± 0.2	53.5 ± 0.2	53.1 ± 0.1	53.2 ± 0.3
Mean cell hemoglobin (pg)						
Day 4	18.4 ± 0.1	18.4 ± 0.1	18.6 ± 0.1	18.5 ± 0.1	18.8 ± 0.1	18.5 ± 0.1
Day 23	18.5 ± 0.1	18.4 ± 0.1	18.6 ± 0.1	18.3 ± 0.1	18.8 ± 0.1	18.7 ± 0.2
Week 14	17.1 ± 0.1	17.2 ± 0.2	17.1 ± 0.1	17.0 ± 0.1	16.8 ± 0.1*	16.9 ± 0.1*
Mean cell hemoglobin concentration (g/dL)						
Day 4	29.4 ± 0.1	29.5 ± 0.1	29.5 ± 0.1	29.5 ± 0.1	29.5 ± 0.1	29.4 ± 0.2
Day 23	30.5 ± 0.1	30.5 ± 0.1	30.8 ± 0.2	30.2 ± 0.2	30.8 ± 0.1	30.9 ± 0.4
Week 14	32.0 ± 0.1	32.1 ± 0.2	32.2 ± 0.2	31.7 ± 0.1	31.7 ± 0.2	31.8 ± 0.2
Platelets (10^3/µL)						
Day 4	1,057.9 ± 34.5	1,059.3 ± 20.5	1,079.9 ± 34.8	1,075.1 ± 51.5	1,093.3 ± 49.7	1,083.5 ± 74.8
Day 23	978.7 ± 20.6	991.6 ± 15.3	925.7 ± 79.2	982.9 ± 21.2	1,010.7 ± 26.0	952.0 ± 33.6
Week 14	680.0 ± 18.9	698.9 ± 16.3	715.1 ± 20.6	703.1 ± 18.1	721.3 ± 14.1	713.5 ± 18.6
Leukocytes (10^3/µL)						
Day 4	8.40 ± 0.39	7.78 ± 0.33	8.22 ± 0.26	8.36 ± 0.49	7.59 ± 0.40	7.56 ± 0.53
Day 23	10.28 ± 0.42	10.21 ± 0.47	10.15 ± 0.38	10.48 ± 0.42	11.22 ± 0.23	10.09 ± 0.39
Week 14	10.76 ± 0.31	10.57 ± 0.30	11.08 ± 0.27	11.54 ± 0.22	11.69 ± 0.31	11.52 ± 0.33
Segmented neutrophils (10^3/µL)						
Day 4	0.80 ± 0.08	0.82 ± 0.03	0.87 ± 0.03	0.93 ± 0.06	0.82 ± 0.03	0.86 ± 0.06
Day 23	0.97 ± 0.10	1.03 ± 0.03	1.12 ± 0.07	1.13 ± 0.06	1.26 ± 0.06	1.26 ± 0.15
Week 14	1.20 ± 0.05	1.31 ± 0.04	1.30 ± 0.04	1.37 ± 0.09	1.50 ± 0.07**	1.47 ± 0.05**
Lymphocytes (10^3/µL)						
Day 4	7.32 ± 0.35	6.72 ± 0.32	7.12 ± 0.27	7.16 ± 0.44	6.53 ± 0.39	6.45 ± 0.49
Day 23	9.01 ± 0.45	8.85 ± 0.46	8.73 ± 0.41	9.05 ± 0.35	9.60 ± 0.22	8.44 ± 0.32
Week 14	9.15 ± 0.26	8.88 ± 0.29	9.44 ± 0.22	9.81 ± 0.14	9.82 ± 0.26	9.68 ± 0.30
Monocytes (10^3/µL)						
Day 4	0.23 ± 0.02	0.19 ± 0.02	0.16 ± 0.02	0.23 ± 0.03	0.18 ± 0.01	0.21 ± 0.02
Day 23	0.22 ± 0.02	0.26 ± 0.02	0.22 ± 0.01	0.24 ± 0.02	0.28 ± 0.01	0.28 ± 0.02
Week 14	0.28 ± 0.02	0.22 ± 0.02	0.20 ± 0.02*	0.22 ± 0.02	0.23 ± 0.01	0.23 ± 0.02
Basophils (10^3/µL)						
Day 4	0.031 ± 0.010	0.026 ± 0.006	0.045 ± 0.010	0.030 ± 0.004	0.035 ± 0.006	0.022 ± 0.004
Day 23	0.038 ± 0.007	0.037 ± 0.003	0.040 ± 0.003	0.031 ± 0.002	0.036 ± 0.003	0.040 ± 0.004
Week 14	0.041 ± 0.003	0.044 ± 0.008	0.054 ± 0.003	0.047 ± 0.005	0.060 ± 0.009	0.059 ± 0.007
Eosinophils (10^3/µL)						
Day 4	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.00
Day 23	0.04 ± 0.01	0.04 ± 0.00	0.06 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.06 ± 0.03
Week 14	0.08 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.08 ± 0.01

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Ginseng

	Vehicle Control	1,000 mg/kg	2,000 mg/kg	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
Male (continued)						
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	9	10	10	10	10	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	10.6 ± 0.7	11.0 ± 0.5	10.1 ± 0.7	10.2 ± 0.5	10.1 ± 0.4	10.3 ± 0.5
Day 23	13.6 ± 0.6	13.0 ± 0.4	13.4 ± 0.3	13.1 ± 0.4	12.8 ± 0.4	13.4 ± 0.4
Week 14	14.6 ± 0.5	13.2 ± 0.4	12.9 ± 0.2*	12.8 ± 0.3*	13.0 ± 0.4	13.0 ± 0.4
Creatinine (mg/dL)						
Day 4	0.45 ± 0.02	0.44 ± 0.02	0.42 ± 0.01	0.42 ± 0.01	0.44 ± 0.02	0.41 ± 0.01
Day 23	0.50 ± 0.00	0.50 ± 0.00	0.51 ± 0.01	0.54 ± 0.02*	0.50 ± 0.00	0.51 ± 0.01
Week 14	0.50 ± 0.00	0.51 ± 0.01	0.50 ± 0.01	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00
Total protein (g/dL)						
Day 4	5.7 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.6 ± 0.1
Day 23	6.5 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.5 ± 0.1
Week 14	6.6 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.0	6.6 ± 0.0	6.7 ± 0.0
Albumin (g/dL)						
Day 4	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.1 ± 0.0	4.2 ± 0.0	4.1 ± 0.1
Day 23	4.5 ± 0.0	4.4 ± 0.0	4.5 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.1
Week 14	4.4 ± 0.0	4.4 ± 0.0	4.5 ± 0.0	4.5 ± 0.0	4.4 ± 0.0	4.4 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	45 ± 2	48 ± 1	47 ± 1	48 ± 1	48 ± 2	46 ± 1
Day 23	36 ± 0	38 ± 1	37 ± 1	37 ± 1	37 ± 1	37 ± 1
Week 14	54 ± 4	51 ± 2	42 ± 2**	39 ± 1**	40 ± 1**	40 ± 2**
Alkaline phosphatase (IU/L)						
Day 4	679 ± 15	687 ± 13	703 ± 12	693 ± 20	704 ± 16	655 ± 13
Day 23	515 ± 10	504 ± 10	509 ± 9	510 ± 16	499 ± 8	487 ± 13
Week 14	198 ± 5	196 ± 7	189 ± 5	183 ± 5	165 ± 2**	171 ± 4**
Creatine kinase (IU/L)						
Day 4	253 ± 28	270 ± 30	243 ± 22	269 ± 36	252 ± 13	256 ± 18
Day 23	162 ± 16	189 ± 20	247 ± 38	216 ± 28	273 ± 39	183 ± 31
Week 14	143 ± 25	141 ± 23	132 ± 15	187 ± 51	168 ± 67	142 ± 19
Sorbitol dehydrogenase (IU/L)						
Day 4	9 ± 0	9 ± 1	8 ± 1	8 ± 1	8 ± 1	8 ± 1
Day 23	13 ± 1	15 ± 1	14 ± 1	15 ± 1	14 ± 1	12 ± 1
Week 14	18 ± 1	18 ± 1	15 ± 1*	12 ± 1**	12 ± 1**	14 ± 1**
Bile acids (μmol/L)						
Day 4	8.2 ± 1.3	5.8 ± 0.9	6.5 ± 1.0	8.3 ± 1.3	6.4 ± 1.2	7.8 ± 1.3
Day 23	3.4 ± 0.6	5.8 ± 1.1	2.9 ± 0.5	4.1 ± 0.8	3.1 ± 0.5	2.7 ± 0.4
Week 14	3.9 ± 0.2	6.9 ± 1.8	4.6 ± 0.7	4.7 ± 0.6	6.1 ± 0.7	4.9 ± 0.7
Corticosterone (ng/mL)						
Day 4	46 ± 15	69 ± 38	47 ± 21	38 ± 15	29 ± 12	43 ± 9
Day 23	191 ± 59	140 ± 54	69 ± 38*	68 ± 24	62 ± 37	191 ± 67
Week 14	43 ± 13	38 ± 10	44 ± 13	33 ± 11	46 ± 6	56 ± 12

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Ginseng

	Vehicle Control	1,000 mg/kg	2,000 mg/kg	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
Female						
Hematology						
n						
Day 4	10	10	9	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	9
Hematocrit (%)						
Day 4	44.9 ± 0.5	46.8 ± 0.4	43.3 ± 2.0	46.0 ± 0.6	45.9 ± 0.2	45.8 ± 0.7
Day 23	47.0 ± 0.3	48.2 ± 0.5	47.9 ± 0.4	47.5 ± 0.5	47.3 ± 0.4	47.9 ± 0.5
Week 14	46.1 ± 0.4	46.6 ± 0.3	46.1 ± 0.4	46.1 ± 0.2	45.6 ± 0.2	46.2 ± 0.5
Hemoglobin (g/dL)						
Day 4	13.6 ± 0.2	14.3 ± 0.1*	13.2 ± 0.6	13.9 ± 0.2	14.0 ± 0.1	13.9 ± 0.2
Day 23	15.0 ± 0.1	15.1 ± 0.2	15.1 ± 0.1	14.9 ± 0.1	14.9 ± 0.1	15.0 ± 0.1
Week 14	14.9 ± 0.1	15.1 ± 0.1	14.9 ± 0.1	14.8 ± 0.1	14.6 ± 0.1	14.8 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	7.31 ± 0.08	7.62 ± 0.07	7.13 ± 0.32	7.54 ± 0.09	7.52 ± 0.04	7.49 ± 0.13
Day 23	8.03 ± 0.05	8.14 ± 0.09	8.17 ± 0.07	8.12 ± 0.07	8.07 ± 0.06	8.17 ± 0.08
Week 14	8.41 ± 0.06	8.51 ± 0.06	8.41 ± 0.05	8.37 ± 0.04	8.38 ± 0.04	8.46 ± 0.10
Mean cell volume (fL)						
Day 4	61.4 ± 0.3	61.4 ± 0.2	60.7 ± 0.3	61.0 ± 0.2	61.0 ± 0.2	61.2 ± 0.2
Day 23	58.5 ± 0.3	59.3 ± 0.2	58.6 ± 0.2	58.4 ± 0.3	58.7 ± 0.3	58.6 ± 0.2
Week 14	54.8 ± 0.2	54.8 ± 0.2	54.8 ± 0.3	55.1 ± 0.1	54.5 ± 0.3	54.6 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	18.6 ± 0.1	18.7 ± 0.1	18.6 ± 0.1	18.4 ± 0.1	18.6 ± 0.1	18.6 ± 0.1
Day 23	18.6 ± 0.1	18.6 ± 0.1	18.5 ± 0.1	18.3 ± 0.1**	18.4 ± 0.1*	18.4 ± 0.1*
Week 14	17.7 ± 0.1	17.7 ± 0.0	17.7 ± 0.1	17.6 ± 0.0	17.5 ± 0.1*	17.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	30.3 ± 0.2	30.5 ± 0.3	30.6 ± 0.1	30.2 ± 0.1	30.4 ± 0.2	30.4 ± 0.1
Day 23	31.9 ± 0.2	31.3 ± 0.1	31.6 ± 0.1	31.3 ± 0.2	31.4 ± 0.1	31.4 ± 0.1
Week 14	32.3 ± 0.1	32.3 ± 0.1	32.3 ± 0.1	32.0 ± 0.1	32.1 ± 0.1	31.9 ± 0.1
Platelets (10^3/µL)						
Day 4	1,138.8 ± 54.8	1,127.0 ± 52.0	1,027.0 ± 116.1	1,185.7 ± 22.6	1,235.4 ± 49.8	1,136.9 ± 45.2
Day 23	967.6 ± 18.1	853.1 ± 50.5	1,006.3 ± 14.8	947.7 ± 16.8	941.4 ± 19.8	902.4 ± 23.5
Week 14	758.8 ± 12.6	745.6 ± 17.7	715.7 ± 21.8	787.6 ± 41.9	833.5 ± 18.4	790.4 ± 37.2
Leukocytes (10^3/µL)						
Day 4	9.42 ± 0.20	10.04 ± 0.36	8.60 ± 0.76	9.33 ± 0.35	9.89 ± 0.31	8.84 ± 0.47
Day 23	10.07 ± 0.29	10.50 ± 0.50	10.68 ± 0.35	10.37 ± 0.43	10.32 ± 0.37	10.53 ± 0.54
Week 14	7.79 ± 0.20	8.10 ± 0.39	7.96 ± 0.35	8.02 ± 0.47	7.54 ± 0.33	7.54 ± 0.54
Segmented neutrophils (10^3/µL)						
Day 4	0.82 ± 0.03	0.93 ± 0.07	0.78 ± 0.09	0.84 ± 0.03	0.86 ± 0.03	0.86 ± 0.05
Day 23	0.99 ± 0.07	1.06 ± 0.06	1.14 ± 0.10	1.00 ± 0.06	1.10 ± 0.08	1.06 ± 0.07
Week 14	1.00 ± 0.05	1.26 ± 0.13	1.13 ± 0.10	1.21 ± 0.07	1.35 ± 0.11*	1.19 ± 0.29
Lymphocytes (10^3/µL)						
Day 4	8.33 ± 0.21	8.84 ± 0.31	7.54 ± 0.66	8.22 ± 0.33	8.73 ± 0.30	7.74 ± 0.41
Day 23	8.75 ± 0.25	9.12 ± 0.45	9.20 ± 0.29	9.07 ± 0.37	8.88 ± 0.38	9.14 ± 0.48
Week 14	6.48 ± 0.18	6.54 ± 0.34	6.55 ± 0.28	6.51 ± 0.40	5.91 ± 0.30	6.10 ± 0.36
Monocytes (10^3/µL)						
Day 4	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.02	0.21 ± 0.02	0.23 ± 0.01	0.19 ± 0.01
Day 23	0.23 ± 0.03	0.23 ± 0.02	0.26 ± 0.02	0.22 ± 0.02	0.24 ± 0.03	0.23 ± 0.02
Week 14	0.22 ± 0.01	0.22 ± 0.02	0.17 ± 0.03	0.20 ± 0.03	0.19 ± 0.02	0.17 ± 0.04
Basophils (10^3/µL)						
Day 4	0.035 ± 0.005	0.035 ± 0.003	0.031 ± 0.005	0.033 ± 0.004	0.039 ± 0.003	0.030 ± 0.005
Day 23	0.032 ± 0.003	0.033 ± 0.003	0.035 ± 0.004	0.028 ± 0.002	0.032 ± 0.004	0.034 ± 0.005
Week 14	0.027 ± 0.004	0.034 ± 0.009	0.027 ± 0.004	0.025 ± 0.005	0.029 ± 0.003	0.026 ± 0.005
Eosinophils (10^3/µL)						
Day 4	0.04 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Day 23	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Week 14	0.06 ± 0.00	0.05 ± 0.01	0.08 ± 0.03	0.08 ± 0.02	0.05 ± 0.00	0.05 ± 0.01

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Ginseng

	Vehicle Control	1,000 mg/kg	2,000 mg/kg	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
Female (continued)						
Clinical Chemistry						
n						
Day 4	10	10	10	10	9	10
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	10.3 ± 0.5	10.6 ± 0.6	9.5 ± 0.6	9.7 ± 0.4	9.1 ± 0.5	10.0 ± 0.3[b]
Day 23	14.4 ± 0.3	14.8 ± 0.7	14.5 ± 0.6	14.0 ± 0.4	13.7 ± 0.6	13.6 ± 0.4
Week 14	12.1 ± 0.4	13.7 ± 0.4*	13.0 ± 0.3	12.7 ± 0.3	12.7 ± 0.3	13.1 ± 0.6
Creatinine (mg/dL)						
Day 4	0.42 ± 0.01	0.42 ± 0.01	0.40 ± 0.00	0.40 ± 0.00	0.42 ± 0.01	0.44 ± 0.02
Day 23	0.50 ± 0.00	0.50 ± 0.01	0.49 ± 0.01	0.49 ± 0.01	0.47 ± 0.02	0.50 ± 0.00
Week 14	0.51 ± 0.01	0.51 ± 0.01	0.51 ± 0.01	0.50 ± 0.00	0.50 ± 0.00	0.51 ± 0.01
Total protein (g/dL)						
Day 4	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.0	5.9 ± 0.1	5.9 ± 0.1	5.9 ± 0.1[b]
Day 23	6.3 ± 0.1	6.5 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1
Week 14	6.6 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.6 ± 0.1
Albumin (g/dL)						
Day 4	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.0[b]
Day 23	4.5 ± 0.1	4.6 ± 0.0	4.5 ± 0.1	4.6 ± 0.0	4.5 ± 0.0	4.5 ± 0.0
Week 14	4.7 ± 0.1	5.0 ± 0.1	5.0 ± 0.0	4.7 ± 0.1	4.6 ± 0.1	4.7 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	40 ± 1	41 ± 1	38 ± 1	40 ± 1	40 ± 2	40 ± 1
Day 23	29 ± 1	27 ± 1	28 ± 1	28 ± 1	29 ± 1	29 ± 1
Week 14	40 ± 2	44 ± 3	33 ± 1**	34 ± 1*	34 ± 2*	33 ± 1*
Alkaline phosphatase (IU/L)						
Day 4	550 ± 15	569 ± 13	530 ± 13	554 ± 13	553 ± 21	546 ± 12
Day 23	353 ± 7	374 ± 8	337 ± 12	371 ± 7	361 ± 7	361 ± 13
Week 14	163 ± 3	146 ± 6	139 ± 5**	146 ± 6	144 ± 6*	142 ± 5*
Creatine kinase (IU/L)						
Day 4	210 ± 21	182 ± 17	243 ± 33	228 ± 15	189 ± 12	216 ± 16
Day 23	177 ± 24	176 ± 28	195 ± 27	195 ± 19	205 ± 25	207 ± 15
Week 14	141 ± 25	91 ± 12	119 ± 28	123 ± 16	110 ± 17	107 ± 12
Sorbitol dehydrogenase (IU/L)						
Day 4	7 ± 0	8 ± 1	7 ± 0	8 ± 1	7 ± 0	8 ± 0
Day 23	12 ± 1	13 ± 1	13 ± 1	12 ± 1	12 ± 1	14 ± 1
Week 14	12 ± 1	13 ± 1	10 ± 1	10 ± 1	10 ± 1	11 ± 1
Bile acids (μmol/L)						
Day 4	4.9 ± 0.6	7.5 ± 0.8	4.7 ± 0.6	4.4 ± 0.4	5.2 ± 1.3	4.4 ± 1.1[b]
Day 23	6.3 ± 1.4	7.9 ± 0.8	5.9 ± 0.8	3.7 ± 0.7	4.9 ± 0.6	3.1 ± 0.2*
Week 14	13.1 ± 2.8	14.4 ± 2.8	5.0 ± 0.9**	4.7 ± 0.9**	4.9 ± 0.6**	4.8 ± 0.6**
Corticosterone (ng/mL)						
Day 4	17 ± 5	34 ± 22	26 ± 7	44 ± 12	64 ± 23[c]	37 ± 11
Day 23	164 ± 41	85 ± 22	120 ± 47	136 ± 44	88 ± 28	177 ± 53
Week 14	120 ± 39	136 ± 51	138 ± 46	82 ± 25	82 ± 28	92 ± 19

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=10

TABLE F2
Hematology Data for Mice in the 3-Month Gavage Study of Ginseng[a]

	Vehicle Control	1,000 mg/kg	2,000 mg/kg	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
Male						
n	10	10	10	10	10	10
Hematocrit (%)	47.4 ± 0.5	48.7 ± 0.6	49.2 ± 0.6	47.1 ± 0.5	48.4 ± 0.8	47.5 ± 0.7
Hemoglobin (g/dL)	15.8 ± 0.2	16.3 ± 0.2	16.5 ± 0.2	15.8 ± 0.2	16.2 ± 0.3	16.0 ± 0.2
Erythrocytes (10^6/µL)	10.61 ± 0.13	10.98 ± 0.11	11.04 ± 0.15	10.52 ± 0.12	10.87 ± 0.18	10.65 ± 0.11
Mean cell volume (fL)	44.7 ± 0.3	44.4 ± 0.2	44.6 ± 0.2	44.8 ± 0.2	44.5 ± 0.1	44.6 ± 0.3
Mean cell hemoglobin (pg)	15.0 ± 0.1	14.9 ± 0.1	14.9 ± 0.1	15.0 ± 0.0	14.9 ± 0.1	15.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.5 ± 0.1	33.6 ± 0.2	33.5 ± 0.1	33.5 ± 0.1	33.5 ± 0.2	33.7 ± 0.2
Platelets (10^3/µL)	1,060.5 ± 67.2	945.6 ± 55.1	986.0 ± 36.6	1,129.8 ± 54.8	963.0 ± 57.0	1,095.8 ± 62.5
Leukocytes (10^3/µL)	5.39 ± 0.48	5.37 ± 0.37	5.05 ± 0.37	5.67 ± 0.52	5.38 ± 0.21	5.21 ± 0.33
Segmented neutrophils (10^3/µL)	0.82 ± 0.09	0.73 ± 0.03	0.79 ± 0.10	0.79 ± 0.09	0.74 ± 0.06	0.70 ± 0.09
Lymphocytes (10^3/µL)	4.37 ± 0.38	4.37 ± 0.34	4.05 ± 0.33	4.63 ± 0.45	4.37 ± 0.17	4.28 ± 0.25
Monocytes (10^3/µL)	0.08 ± 0.02	0.13 ± 0.02	0.09 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.10 ± 0.01
Basophils (10^3/µL)	0.009 ± 0.002	0.011 ± 0.002	0.011 ± 0.003	0.015 ± 0.002	0.011 ± 0.003	0.011 ± 0.002
Eosinophils (10^3/µL)	0.11 ± 0.02	0.14 ± 0.03	0.11 ± 0.02	0.11 ± 0.01	0.15 ± 0.02	0.11 ± 0.02
Female						
n	9	10	10	9	10	10
Hematocrit (%)	49.5 ± 0.5	48.8 ± 1.0	47.2 ± 0.9	47.2 ± 0.9	49.5 ± 1.0	48.6 ± 0.9
Hemoglobin (g/dL)	16.8 ± 0.2	16.6 ± 0.3	16.2 ± 0.3	16.2 ± 0.3	17.1 ± 0.3	16.7 ± 0.3
Erythrocytes (10^6/µL)	11.13 ± 0.13	10.84 ± 0.21	10.68 ± 0.20	10.64 ± 0.19	11.23 ± 0.23	10.96 ± 0.23
Mean cell volume (fL)	44.5 ± 0.3	45.0 ± 0.2	44.2 ± 0.2	44.4 ± 0.2	44.0 ± 0.2	44.4 ± 0.2
Mean cell hemoglobin (pg)	15.1 ± 0.1	15.4 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.3 ± 0.1	15.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.1 ± 0.2	34.1 ± 0.2	34.4 ± 0.2	34.4 ± 0.1	34.6 ± 0.2	34.3 ± 0.1
Platelets (10^3/µL)	700.1 ± 40.6	783.5 ± 54.9	769.4 ± 45.1	694.1 ± 62.0	663.2 ± 52.9	653.5 ± 37.9
Leukocytes (10^3/µL)	3.57 ± 0.15	3.76 ± 0.28	4.27 ± 0.46	3.49 ± 0.32	3.39 ± 0.30	3.58 ± 0.28
Segmented neutrophils (10^3/µL)	0.39 ± 0.02	0.44 ± 0.05	0.40 ± 0.06	0.35 ± 0.05	0.37 ± 0.05	0.37 ± 0.06
Lymphocytes (10^3/µL)	2.98 ± 0.14	3.17 ± 0.22	3.68 ± 0.39	2.99 ± 0.26	2.88 ± 0.27	3.07 ± 0.25
Monocytes (10^3/µL)	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Basophils (10^3/µL)	0.011 ± 0.001	0.013 ± 0.003	0.010 ± 0.002	0.013 ± 0.002	0.010 ± 0.001	0.012 ± 0.002
Eosinophils (10^3/µL)	0.12 ± 0.01	0.08 ± 0.02	0.12 ± 0.02	0.09 ± 0.02	0.09 ± 0.01	0.08 ± 0.02

[a] Mean ± standard error. Statistical tests were performed on unrounded data. Differences from the vehicle control group were not significant by Dunn's test.

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

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Gavage Study of Ginseng[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	175 ± 5	177 ± 4	178 ± 3	181 ± 4	175 ± 5	183 ± 3
Adrenal glands	0.035 ± 0.002	0.034 ± 0.001	0.034 ± 0.001	0.035 ± 0.001	0.032 ± 0.002	0.033 ± 0.002
Absolute	0.203 ± 0.014	0.194 ± 0.009	0.190 ± 0.006	0.193 ± 0.009	0.185 ± 0.013	0.178 ± 0.009
Relative						
Heart						
Absolute	0.64 ± 0.02	0.66 ± 0.02	0.66 ± 0.02	0.68 ± 0.02	0.66 ± 0.02	0.67 ± 0.02
Relative	3.656 ± 0.090	3.746 ± 0.061	3.722 ± 0.073	3.745 ± 0.098	3.744 ± 0.069	3.654 ± 0.119
R. Kidney						
Absolute	0.74 ± 0.02	0.71 ± 0.03	0.74 ± 0.02	0.75 ± 0.02	0.72 ± 0.01	0.80 ± 0.02
Relative	4.259 ± 0.062	4.018 ± 0.062*	4.151 ± 0.045	4.136 ± 0.064	4.095 ± 0.077	4.353 ± 0.044
Liver						
Absolute	9.53 ± 0.16	9.35 ± 0.31	9.76 ± 0.29	10.14 ± 0.26	9.40 ± 0.26	10.48 ± 0.36
Relative	54.597 ± 0.910	52.704 ± 0.813	54.720 ± 0.742	55.916 ± 1.130	53.635 ± 0.816	57.162 ± 1.644
Lung						
Absolute	1.27 ± 0.13	1.61 ± 0.14	1.33 ± 0.11	1.52 ± 0.08	1.23 ± 0.11	1.55 ± 0.24
Relative	7.247 ± 0.660	9.059 ± 0.735	7.474 ± 0.704	8.408 ± 0.496	6.974 ± 0.521	8.448 ± 1.244
R. Testis						
Absolute	0.960 ± 0.029	0.971 ± 0.041	1.004 ± 0.008	1.002 ± 0.016	0.975 ± 0.019	0.977 ± 0.028
Relative	5.492 ± 0.078	5.471 ± 0.138	5.638 ± 0.078	5.534 ± 0.173	5.569 ± 0.139	5.338 ± 0.173
Thymus						
Absolute	0.446 ± 0.015	0.475 ± 0.014	0.446 ± 0.030	0.452 ± 0.027	0.431 ± 0.015	0.450 ± 0.027
Relative	2.550 ± 0.072	2.686 ± 0.095	2.497 ± 0.148	2.493 ± 0.138	2.458 ± 0.054	2.450 ± 0.127
Female						
Necropsy body wt	129 ± 3	133 ± 3	136 ± 6	131 ± 2	130 ± 3	129 ± 3
Adrenal glands						
Absolute	0.044 ± 0.003	0.041 ± 0.003	0.043 ± 0.002	0.044 ± 0.003	0.039 ± 0.003	0.042 ± 0.002
Relative	0.343 ± 0.020	0.310 ± 0.024	0.319 ± 0.010	0.340 ± 0.026	0.297 ± 0.015	0.328 ± 0.015
Heart						
Absolute	0.50 ± 0.01	0.51 ± 0.01	0.53 ± 0.02	0.52 ± 0.01	0.50 ± 0.01	0.51 ± 0.01
Relative	3.887 ± 0.072	3.850 ± 0.081	3.933 ± 0.080	3.990 ± 0.094	3.870 ± 0.069	3.950 ± 0.069
R. Kidney						
Absolute	0.56 ± 0.02	0.56 ± 0.01	0.59 ± 0.01	0.58 ± 0.01	0.57 ± 0.01	0.60 ± 0.02
Relative	4.320 ± 0.143	4.213 ± 0.017	4.384 ± 0.114	4.408 ± 0.055	4.423 ± 0.088	4.647 ± 0.118
Liver						
Absolute	6.05 ± 0.22	6.61 ± 0.27	6.88 ± 0.43	6.70 ± 0.18	6.43 ± 0.12	6.53 ± 0.11
Relative	46.845 ± 1.122	49.605 ± 1.151	50.480 ± 1.843	51.209 ± 1.416	49.580 ± 0.960	50.595 ± 1.863
Lung						
Absolute	1.08 ± 0.07	1.18 ± 0.10	1.14 ± 0.06	1.05 ± 0.04	1.09 ± 0.08	1.21 ± 0.12
Relative	8.356 ± 0.593	8.823 ± 0.650	8.466 ± 0.628	8.028 ± 0.211	8.437 ± 0.794	9.478 ± 1.122
Thymus						
Absolute	0.367 ± 0.021	0.368 ± 0.011	0.402 ± 0.014	0.356 ± 0.017	0.370 ± 0.014	0.384 ± 0.012
Relative	2.842 ± 0.130	2.767 ± 0.055	2.976 ± 0.152	2.726 ± 0.137	2.857 ± 0.118	2.974 ± 0.131

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

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

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of Ginseng[a]

	Vehicle Control	1,000 mg/kg	2,000 mg/kg	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	354 ± 6	365 ± 8	351 ± 5	345 ± 5	354 ± 6	339 ± 8
Heart						
Absolute	1.01 ± 0.02	1.04 ± 0.03	1.03 ± 0.01	0.98 ± 0.03	0.99 ± 0.02	0.99 ± 0.03
Relative	2.841 ± 0.045	2.839 ± 0.039	2.944 ± 0.029	2.835 ± 0.044	2.797 ± 0.037	2.911 ± 0.043
R. Kidney						
Absolute	1.13 ± 0.02	1.15 ± 0.03	1.17 ± 0.02	1.13 ± 0.02	1.20 ± 0.02	1.19 ± 0.03
Relative	3.194 ± 0.035	3.146 ± 0.056	3.336 ± 0.036	3.261 ± 0.029	3.394 ± 0.041**	3.500 ± 0.068**
Liver						
Absolute	13.20 ± 0.29	13.88 ± 0.45	14.00 ± 0.24	12.83 ± 0.31	13.63 ± 0.37	12.87 ± 0.40
Relative	37.250 ± 0.356	38.008 ± 0.577	39.859 ± 0.440**	37.116 ± 0.531	38.504 ± 0.669	37.877 ± 0.538
Lung						
Absolute	1.67 ± 0.07	1.71 ± 0.09	1.77 ± 0.06	1.65 ± 0.06	1.67 ± 0.06	1.61 ± 0.05
Relative	4.709 ± 0.168	4.677 ± 0.197	5.048 ± 0.152	4.768 ± 0.157	4.722 ± 0.169	4.751 ± 0.114
R. Testis						
Absolute	1.506 ± 0.014	1.534 ± 0.036	1.496 ± 0.040	1.500 ± 0.017	1.522 ± 0.023	1.385 ± 0.128
Relative	4.258 ± 0.047	4.211 ± 0.059	4.258 ± 0.103	4.347 ± 0.052	4.308 ± 0.071	4.024 ± 0.351
Thymus						
Absolute	0.306 ± 0.018	0.291 ± 0.010	0.311 ± 0.012	0.302 ± 0.013	0.310 ± 0.016	0.292 ± 0.009
Relative	0.861 ± 0.045	0.798 ± 0.018	0.884 ± 0.028	0.874 ± 0.038	0.877 ± 0.047	0.861 ± 0.019
Female						
Necropsy body wt	206 ± 5	202 ± 4	199 ± 3	205 ± 3	210 ± 5	201 ± 5
Heart						
Absolute	0.68 ± 0.02	0.69 ± 0.02	0.63 ± 0.01	0.67 ± 0.02	0.67 ± 0.02	0.67 ± 0.01
Relative	3.332 ± 0.072	3.443 ± 0.062	3.196 ± 0.071	3.261 ± 0.090	3.209 ± 0.074	3.355 ± 0.065
R. Kidney						
Absolute	0.76 ± 0.02	0.74 ± 0.02	0.72 ± 0.01	0.74 ± 0.01	0.77 ± 0.02	0.75 ± 0.02
Relative	3.690 ± 0.074	3.663 ± 0.095	3.646 ± 0.086	3.634 ± 0.039	3.663 ± 0.072	3.737 ± 0.072
Liver						
Absolute	7.50 ± 0.14	7.43 ± 0.16	7.40 ± 0.12	7.32 ± 0.17	7.60 ± 0.19	7.49 ± 0.11
Relative	36.564 ± 0.722	36.904 ± 0.733	37.295 ± 0.668	35.747 ± 0.622	36.220 ± 0.541	37.318 ± 0.417
Lung						
Absolute	1.19 ± 0.05	1.20 ± 0.06[b]	1.09 ± 0.06	1.25 ± 0.07	1.28 ± 0.05	1.17 ± 0.03
Relative	5.778 ± 0.173	5.916 ± 0.271[b]	5.505 ± 0.301	6.112 ± 0.310	6.103 ± 0.218	5.820 ± 0.169
Thymus						
Absolute	0.254 ± 0.009	0.254 ± 0.014	0.233 ± 0.007	0.259 ± 0.009	0.273 ± 0.011	0.264 ± 0.018
Relative	1.243 ± 0.050	1.255 ± 0.050	1.172 ± 0.035	1.264 ± 0.034	1.297 ± 0.030	1.305 ± 0.066

** Significantly different (P≤0.01) from the vehicle control group by Williams' or Dunnett's test

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

[b] n=9

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Gavage Study of Ginseng[a]

	Vehicle Control	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	25.6 ± 0.3	25.1 ± 0.2	25.6 ± 0.4	25.9 ± 0.4	24.4 ± 0.3*	24.8 ± 0.3
Adrenal glands						
Absolute	0.014 ± 0.002	0.013 ± 0.002	0.012 ± 0.001	0.012 ± 0.002	0.016 ± 0.002	0.014 ± 0.002
Relative	0.546 ± 0.092	0.528 ± 0.091	0.460 ± 0.031	0.480 ± 0.081	0.647 ± 0.090	0.566 ± 0.071
Heart						
Absolute	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.21 ± 0.00	0.19 ± 0.01	0.18 ± 0.01
Relative	7.370 ± 0.187	7.267 ± 0.423	7.336 ± 0.273	8.027 ± 0.141	7.759 ± 0.247	7.231 ± 0.281
R. Kidney						
Absolute	0.25 ± 0.01	0.24 ± 0.00	0.26 ± 0.01	0.25 ± 0.01	0.24 ± 0.00	0.25 ± 0.00
Relative	9.769 ± 0.220	9.598 ± 0.201	10.170 ± 0.162	9.830 ± 0.258	9.716 ± 0.146	10.052 ± 0.170
Liver						
Absolute	1.54 ± 0.04	1.42 ± 0.02	1.51 ± 0.07	1.59 ± 0.05	1.49 ± 0.01	1.48 ± 0.02
Relative	60.102 ± 1.154	56.656 ± 0.611	58.690 ± 2.061	61.431 ± 1.090	61.040 ± 0.801	59.902 ± 0.608
Lung						
Absolute	0.33 ± 0.02	0.31 ± 0.02	0.34 ± 0.02	0.32 ± 0.02	0.31 ± 0.02	0.29 ± 0.01
Relative	12.917 ± 0.893	12.446 ± 0.888	13.228 ± 0.586	12.342 ± 0.740	12.746 ± 0.515	11.640 ± 0.224
R. Testis						
Absolute	0.107 ± 0.003	0.097 ± 0.004	0.108 ± 0.001	0.104 ± 0.003	0.102 ± 0.003	0.111 ± 0.002
Relative	4.163 ± 0.134	3.872 ± 0.168	4.206 ± 0.101	4.010 ± 0.119	4.181 ± 0.142	4.480 ± 0.122
Thymus						
Absolute	0.062 ± 0.005	0.054 ± 0.002	0.063 ± 0.002	0.056 ± 0.005	0.056 ± 0.005	0.062 ± 0.004
Relative	2.426 ± 0.215	2.138 ± 0.055	2.461 ± 0.067	2.173 ± 0.172	2.306 ± 0.216	2.503 ± 0.205
Female						
Necropsy body wt	19.7 ± 0.4	19.7 ± 0.2	19.7 ± 0.3	20.0 ± 0.2	19.7 ± 0.3	19.8 ± 0.2
Adrenal glands						
Absolute	0.014 ± 0.001	0.014 ± 0.001	0.013 ± 0.001	0.014 ± 0.001	0.013 ± 0.001	0.014 ± 0.001
Relative	0.700 ± 0.060	0.721 ± 0.051	0.648 ± 0.041	0.699 ± 0.055	0.641 ± 0.050	0.707 ± 0.045
Heart						
Absolute	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.00
Relative	7.652 ± 0.353	8.094 ± 0.329	8.407 ± 0.493	7.335 ± 0.364	7.896 ± 0.371	7.484 ± 0.207
R. Kidney						
Absolute	0.16 ± 0.00	0.15 ± 0.00	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.01	0.16 ± 0.00
Relative	8.249 ± 0.160	7.770 ± 0.076	7.988 ± 0.168	7.905 ± 0.122	7.957 ± 0.229	8.036 ± 0.146
Liver						
Absolute	1.08 ± 0.04	1.07 ± 0.01	1.08 ± 0.04	1.13 ± 0.03	1.11 ± 0.03	1.12 ± 0.02
Relative	54.844 ± 1.039	54.417 ± 0.416	54.860 ± 1.790	56.220 ± 1.170	56.546 ± 1.242	56.514 ± 0.728
Lung						
Absolute	0.20 ± 0.01	0.26 ± 0.02	0.27 ± 0.02*	0.25 ± 0.01	0.26 ± 0.01	0.27 ± 0.02*
Relative	10.299 ± 0.616	13.059 ± 0.845*	13.415 ± 0.934*	12.465 ± 0.540	13.132 ± 0.537*	13.411 ± 0.765*
Thymus						
Absolute	0.074 ± 0.003	0.070 ± 0.004	0.078 ± 0.004	0.073 ± 0.002	0.068 ± 0.006	0.069 ± 0.002
Relative	3.762 ± 0.133	3.580 ± 0.229	3.967 ± 0.208	3.646 ± 0.091	3.427 ± 0.297	3.468 ± 0.114

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

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

TABLE G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of Ginseng[a]

	Vehicle Control	1,000 mg/kg	2,000 mg/kg	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	35.6 ± 0.8	36.5 ± 0.8	36.0 ± 0.8	37.9 ± 1.4	36.1 ± 0.7	36.5 ± 0.9
Heart						
Absolute	0.20 ± 0.02	0.22 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01
Relative	5.633 ± 0.356	5.918 ± 0.244	5.878 ± 0.180	5.388 ± 0.282	5.470 ± 0.174	5.098 ± 0.191
R. Kidney						
Absolute	0.30 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.28 ± 0.01	0.30 ± 0.00
Relative	8.361 ± 0.182	8.080 ± 0.174	8.305 ± 0.146	8.100 ± 0.237	7.841 ± 0.155	8.286 ± 0.173
Liver						
Absolute	1.65 ± 0.05	1.65 ± 0.05	1.65 ± 0.08	1.77 ± 0.10	1.65 ± 0.04	1.66 ± 0.03
Relative	46.436 ± 1.239	45.254 ± 0.722	45.636 ± 1.294	46.427 ± 1.428	45.722 ± 0.677	45.624 ± 0.929
Lung						
Absolute	0.27 ± 0.02	0.30 ± 0.02	0.31 ± 0.02	0.28 ± 0.02	0.30 ± 0.01	0.26 ± 0.02
Relative	7.444 ± 0.453	8.158 ± 0.507	8.616 ± 0.501	7.251 ± 0.399	8.245 ± 0.429	7.149 ± 0.435
R. Testis						
Absolute	0.121 ± 0.003	0.123 ± 0.001	0.125 ± 0.002	0.124 ± 0.001	0.120 ± 0.004	0.127 ± 0.002
Relative	3.406 ± 0.068	3.392 ± 0.072	3.486 ± 0.060	3.330 ± 0.149	3.332 ± 0.099	3.489 ± 0.071
Thymus						
Absolute	0.040 ± 0.003	0.043 ± 0.003	0.045 ± 0.004	0.062 ± 0.008*	0.048 ± 0.003	0.041 ± 0.004
Relative	1.125 ± 0.085	1.162 ± 0.070	1.263 ± 0.112	1.594 ± 0.172*	1.317 ± 0.084	1.122 ± 0.097
Female						
Necropsy body wt	29.6 ± 1.0	31.7 ± 1.0	30.3 ± 0.8	31.0 ± 0.8	28.1 ± 0.9	28.5 ± 0.7
Heart						
Absolute	0.14 ± 0.00	0.15 ± 0.00	0.15 ± 0.01	0.14 ± 0.00	0.14 ± 0.01	0.15 ± 0.00
Relative	4.901 ± 0.232	4.643 ± 0.208	4.809 ± 0.133	4.643 ± 0.117	5.153 ± 0.191	5.217 ± 0.114
R. Kidney						
Absolute	0.17 ± 0.00	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.00	0.17 ± 0.00	0.18 ± 0.01
Relative	5.852 ± 0.180	5.903 ± 0.171	6.002 ± 0.134	5.815 ± 0.141	6.173 ± 0.149	6.247 ± 0.178
Liver						
Absolute	1.31 ± 0.04	1.41 ± 0.04	1.36 ± 0.05	1.34 ± 0.04	1.27 ± 0.04	1.31 ± 0.05
Relative	44.452 ± 0.572	44.753 ± 0.785	44.762 ± 0.921	43.324 ± 0.992	45.243 ± 0.749	45.752 ± 0.865
Lung						
Absolute	0.34 ± 0.01	0.33 ± 0.02	0.30 ± 0.02	0.30 ± 0.01	0.30 ± 0.01	0.32 ± 0.01
Relative	11.463 ± 0.433	10.364 ± 0.378	9.926 ± 0.608	9.685 ± 0.487	10.735 ± 0.570	11.202 ± 0.448
Thymus						
Absolute	0.055 ± 0.003	0.059 ± 0.002	0.057 ± 0.003	0.057 ± 0.003	0.053 ± 0.003	0.053 ± 0.005[b]
Relative	1.867 ± 0.093	1.864 ± 0.073	1.907 ± 0.113	1.839 ± 0.079	1.882 ± 0.099	1.845 ± 0.142[b]

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test

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

[b] n=9

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

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

	Vehicle Control	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
n	10	10	10	9
Weights (g)				
Necropsy body wt	354 ± 6	345 ± 5	354 ± 6	345.7 ± 5
L. Cauda epididymis	0.1445 ± 0.0031	0.1447 ± 0.0043	0.1471 ± 0.0037	0.1454 ± 0.0032
L. Epididymis	0.4491 ± 0.0088	0.4400 ± 0.0049	0.4550 ± 0.0053	0.4516 ± 0.0059
L. Testis	1.6959 ± 0.0657	1.5979 ± 0.0471	1.5704 ± 0.0244	1.6668 ± 0.0525
Spermatid measurement				
Spermatid heads (103/mg testis)	128.80 ± 3.19	127.63 ± 3.40	139.12 ± 2.57	128.59 ± 6.47
Spermatid heads (106/testis)	179.88 ± 5.13	176.38 ± 3.98	190.88 ± 3.28	173.89 ± 7.16
Epididymal spermatozoal measurements				
Sperm motility (%)	90.76 ± 0.65	89.81 ± 0.63	89.44 ± 0.75	89.24 ± 0.76
Sperm (106/cauda epididymis)	87 ± 4	78 ± 5	86 ± 6	74 ± 4
Sperm (103/mg cauda epididymis)	603 ± 32	542 ± 35	586 ± 38	504 ± 20

[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 and epididymal spermatozoal measurements).

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of Ginseng[a]

	Vehicle Control	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	206 ± 5	205 ± 3	210 ± 5	201 ± 5
Proportion of regular cycling females[b]	8/9	2/2	5/6	2/2
Estrous cycle length (days)	4.8 ± 0.24[c]	5.0 ± 0.00[d]	5.1 ± 0.42[e]	5.5 ± 0.50[d]
Estrous stages (% of cycle)				
Diestrus	57.5	44.2	49.2	48.3
Proestrus	6.7	8.3	3.3	3.3
Estrus	25.8	18.3	25.8	23.3
Metestrus	1.7	1.7	0.8	2.5
Uncertain diagnoses	8.3	27.5	20.8	22.5

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices among dose groups and between the vehicle control group and each dosed group indicated the dosed females did not have extended estrus or diestrus.

[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.

[d] Estrous cycle was longer than 12 days or unclear in 8 of 10 animals.

[e] Estrous cycle was longer than 12 days or unclear in 4 of 10 animals.

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

	Vehicle Control	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	35.6 ± 0.8	37.9 ± 1.4	36.1 ± 0.7	36.5 ± 0.9
L. Cauda epididymis	0.163 ± 0.0011	0.0166 ± 0.0006	0.0139 ± 0.0009	0.0156 ± 0.0007
L. Epididymis	0.0454 ± 0.0011	0.0468 ± 0.0011	0.0411 ± 0.0017	0.0461 ± 0.0010
L. Testis	0.1147 ± 0.0020	0.1183 ± 0.0010	0.1165 ± 0.0055	0.1204 ± 0.0014
Spermatid measurement				
Spermatid heads (103/mg testis)	169.48 ± 6.15	187.16 ± 4.69	185.13 ± 8.65	181.37 ± 5.70
Spermatid heads (106/testis)	18.65 ± 0.91	20.99 ± 0.39	19.77 ± 0.75	20.89 ± 0.55
Epididymal spermatozoal measurements				
Sperm motility (%)	92.01 ± 0.45	91.23 ± 0.53	91.83 ± 0.42	91.34 ± 0.44
Sperm (106/cauda epididymis)	15.3 ± 1.2	16.6 ± 1.4	17.8 ± 1.9	17.9 ± 1.8
Sperm (103/mg cauda epididymis)	978.7 ± 98.6	1,003.7 ± 79.7	1,289.9 ± 117.0	1,161.6 ± 121.2

[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 and epididymal spermatozoal measurements).

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of Ginseng[a]

	Vehicle Control	3,000 mg/kg	4,000 mg/kg	5,000 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	29.6 ± 1.0	31.0 ± 0.8	28.1 ± 0.9	28.5 ± 0.7
Proportion of regular cycling females[b]	6/9	6/8	1/2	3/4
Estrous cycle length (days)	4.1 ± 0.09[c]	4.6 ± 0.74[c]	4.3 ± 0.25[d]	4.9 ± 0.24[e]
Estrous stages (% of cycle)				
Diestrus	37.5	24.2	25.0	23.3
Proestrus	0.0	0.0	0.0	0.0
Estrus	37.5	47.5	45.0	40.8
Metestrus	20.8	17.5	19.2	21.7
Uncertain diagnoses	4.2	10.8	10.8	14.2

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. The tests for equality of transition probability matrices among dose groups and between the vehicle control group and each dosed group indicated the dosed females did not have extended estrus or diestrus.

[b] Number of females with a regular cycle/number of females cycling

[c] Estrous cycle was longer than 12 days or unclear in 3 of 10 animals.

[d] Estrous cycle was longer than 12 days or unclear in 8 of 10 animals.

[e] Estrous cycle was longer than 12 days or unclear in 6 of 10 animals.

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Ginseng

Ginseng extract from the plant *Panax ginseng* C.A. Meyer was obtained from Extracts Plus, Inc. (Vista, CA), in two lots (3021261 and 302500702) and from Plus Pharma, Inc. (Vista, CA), in one lot (3031978). Lot 3021261 was used during the 2-week studies, lot 302500702 was used during the 3-month studies, and lot 3031978 was used during the 2-year studies. All lots were produced by extracting ginseng root with 80% aqueous ethanol. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Laboratories, Chemistry Support Services (Columbus, OH). The study laboratory at Battelle Columbus Operations (Columbus, OH) confirmed the identity of lots 3021261 and 302500702 using infrared (IR) spectroscopy. Multiple standardized analyses of all three lots were performed by Covance Laboratories, Inc. (Madison, WI), and proton-induced X-ray emission (PIXE) spectroscopy was performed on each lot by Element Analysis Corporation (Lexington, KY). Reports on analyses performed in support of the ginseng studies are on file at the National Institute of Environmental Health Sciences.

IR spectrometry was used to obtain benchmark fingerprints for each lot, and spectra were compared between lots; spectra were similar for all lots. All lots of the chemical, a light tan powder, were characterized as ginseng by weight loss on drying; analyses by Covance Laboratories, Inc., for vitamin, total protein, ash, total fatty acid, total fat, total dietary fiber, amino acid, chloride, metal, organophosphate pesticide, and nitrosamine content; PIXE analysis for common elements other than carbon, hydrogen, and oxygen; ion chromatography for quantitation of selected anions; total carbohydrate and ethanol-soluble carbohydrate analysis; high-performance liquid chromatography (HPLC) with refractive index detection for mono- and disaccharide analysis and quantitation of selected sugars; and size exclusion chromatography to determine the molecular weight distribution of the glycans. A representative IR spectrum is presented in Figure I1.

The purity of each lot of ginseng was determined by the analytical chemistry laboratory based on the profile of ginsenosides in the test material using methodologies based on the American Botanical Council (2001), which was a comprehensive study of ginseng products. Solutions of *Panax ginseng* C.A. Meyer were analyzed and compared to authentic standards (ChromaDex Corporate, Irvine, CA) of available ginsenosides using HPLC by systems A and B (Table I1).

System A was optimized for the separation of the ginsenosides and system B was optimized to obtain the overall purity profile of the test material. Weight percentages of the ginsenosides in the test material were determined using the method of standard addition and analysis by system A (Table I2). These values were consistent with those from the Certificate of Analysis for these lots.

Additional components of the test material were characterized by the analytical chemistry laboratory using reverse phase HPLC with mass spectrometric (MS) detection and positive electrospray ionization to confirm the identity of ginsenosides and gas chromatographic analyses with flame ionization and/or MS detection to identify six, 13, or 12 non-ginsenoside components of lots 3021261, 302500702, and 3031978, respectively.

Taken together, these data indicate that all three lots of the test material were ginseng and their composition was consistent with the expected composition of typical ginseng.

Stability studies of lot 3021261 of the bulk chemical were performed by the analytical chemistry laboratory using HPLC by system C (Table I1). These studies indicated that ginseng was stable as a bulk chemical for at least 14 days when stored protected from light in sealed glass containers at temperatures up to 25° C. At 60° C, some physical changes to the sample were evident as well as slight changes in the chromatographic profile of three of the individual ginsenosides. To ensure stability, lots 3021261 and 302500702 were stored at room temperature in sealed amber glass bottles containing a headspace of argon gas, and lot 3031978 was stored at room temperature in double plastic bags under an argon headspace, sealed with tape, inside sealed plastic drums also containing an argon

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

Methylcellulose

For the 2-week studies, methylcellulose was obtained from Spectrum Quality Products (Gardena, CA) in one lot (QG1176). The identity of lot QC1176 was confirmed by the study laboratory using IR spectroscopy; the spectrum was consistent with the structure of methylcellulose. The average methoxyl content determined by Galbraith Laboratories, Inc., was 31.3%.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing ginseng with 0.5% methylcellulose (2-week studies) or sterile water (3-month and 2-year studies) to give the required concentrations (Table I3). The vehicle for the 2-week study was selected as 0.5% methylcellulose with the anticipation that higher doses might be needed in the 3-month and 2-year studies, which in methylcellulose might form suspensions rather than solutions. During the 2-week studies, methylcellulose demonstrated a tendency to form microbial growth after 2 weeks. Further work for the 3-month and 2-year studies showed that sterile water provided better control of microbial growth and accommodated higher formulation concentrations. The dose formulations for the 2-week studies were stored at approximately 5° C in amber glass bottles sealed with Teflon®-lined lids for up to 9 days. The dose formulations for the 3-month and 2-year studies were stored at less than or equal to –20° C in sealed Nalgene® plastic bottles for up to 39 or 44 days, respectively.

The analytical chemistry laboratory determined that a 400 mg/mL dose formulation in 0.5% methylcellulose was gavagable using 16- to 25-gauge needles.

Stability studies of a 2.5 mg/mL formulation in 0.5% methylcellulose or sterile water were conducted by the analytical chemistry laboratory and the study laboratory, respectively; all analyses used HPLC by system C. Homogeneity of the dose formulations in both vehicles was confirmed. Stability of dose formulation in 0.5% methylcellulose was confirmed for up to 42 days in sealed amber glass bottles at room temperature or 5° C, and for up to 3 hours under simulated animal room conditions. Stability of dose formulations in sterile water was confirmed for up to 45 days in sealed plastic bottles protected from light at room temperature, 5° C, and at less than or equal to –20° C, but microbial growth occurred in samples stored at the two higher temperatures.

Periodic analyses of the dose formulations of ginseng were conducted by the study laboratory using HPLC by system C; this system separated the seven ginsenosides; Rg1 was selected as the appropriate marker for formulation analysis (Table I2). During the 2-week studies, the dose formulations were analyzed once; all six of the dose formulations analyzed were within 10% of the target concentrations (Table I4). Animal room samples of these dose formulations were also analyzed; four of five rat animal room samples and all five mouse animal room samples were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table I5). Of the dose formulations analyzed, all 26 were within 10% of the target concentrations; all 16 rat and 15 mouse animal room samples were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 3 months (Table I6). Of the dose formulations analyzed, 75 of 76 were within 10% of the target concentrations; all nine rat and all nine mouse animal room samples were within 10% of the target concentrations.

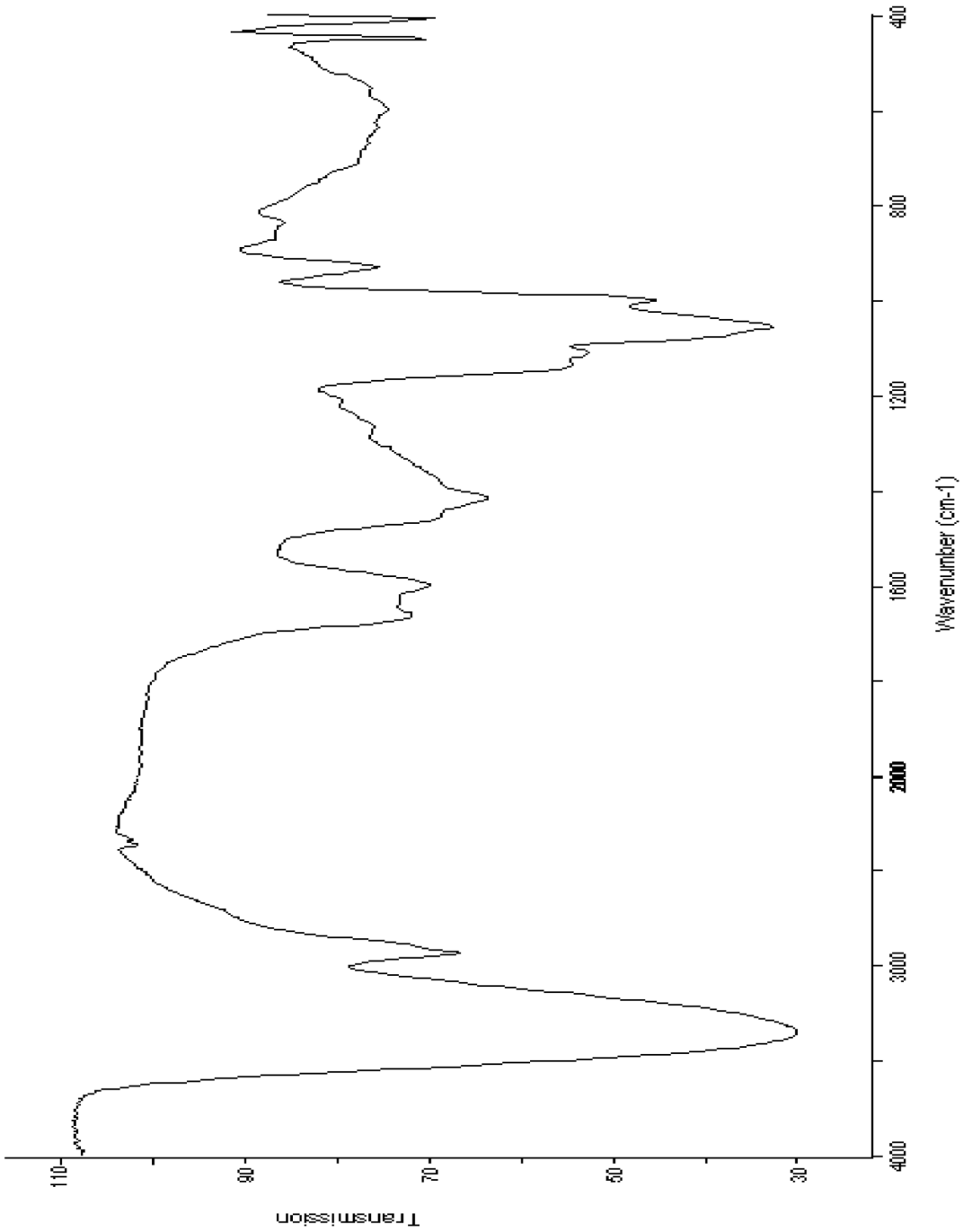

FIGURE I1
Infrared Absorption Spectrum of Ginseng

TABLE I1
High-Performance Liquid Chromatography Systems Used in the Gavage Studies of Ginseng[a]

Detection System	Column	Solvent System
System A		
Ultraviolet (205 nm) light	Jupiter® C18 or C18 (2), 250 mm × 4.6 mm, 5 µm particle size (Phenomenex, Inc., Torrance, CA)	A) 95:5 Milli-Q® water:acetonitrile and B) 10:90 Milli-Q® water:acetonitrile; 88% A:12% B then linear gradient to 60% A:40% B in 40 minutes, held 10 minutes, then linear gradient to 88% A:12% B in 1 minute, then held for 14 minutes; flow rate 1.5 mL/minute
System B		
Ultraviolet photodiode array scanning from 190 to 350 nm or with monitoring at 205 nm	Polaris® C18-A, 250 mm × 4.6 mm, 5 µm particle size (Varian, Inc., Lake Forest, CA)	A) 99:1 Milli-Q® water:acetonitrile and B) 50:50 Milli-Q® water:acetonitrile and C) 10:90 Milli-Q® water:acetonitrile; 100% A for 5 minutes, then linear gradient to 100% B in 40 minutes, then linear gradient to 100% C in 0.1 minutes, held for 14.9 minutes, then linear gradient to 100% A in 0.1 minute, then held for 19.9 minutes; flow rate 1.4 mL/minute
System C		
Ultraviolet (205 nm) light	Polaris™ C18-A, 250 mm × 4.6 mm, 5 µm particle size (MetaChem Technologies, Inc., Lake Forest, CA.	A) 84:16 Milli-Q® water:acetonitrile and B) 64:36 Milli-Q® water:acetonitrile; linear gradient from 100% A to 100% B in 40 minutes, held for 10 minutes, then linear gradient to 100% A in 1 minute, then held for 9 minutes; flow rate 1.5 mL/minute

[a] The high-performance liquid chromatographs were manufactured by Waters Corporation (Milford, MA) (Systems A and B) or Agilent Technologies (Palo Alto, CA) (System C).

TABLE I2
Major Ginsenosides in the Test Chemical Used in the Gavage Studies of Ginseng[a]

Lot No.	Rg1	Re	Rf	Rb1	Rc	Rb2	Rd	Total Ginsenosides
3021261	2.2	0.7	0.3	1.7	1.0	0.8	0.7	7.4
302500702	1.4	1.6	0.4	3.4	1.5	1.8	0.8	10.9
3031978	1.5	1.4	0.4	1.6	1.0	0.9	0.6	7.4

[a] All values are weight percentages

TABLE I3
Preparation and Storage of Dose Formulations in the Gavage Studies of Ginseng

2-Week Studies	3-Month Studies	2-Year Studies
Preparation		
The dosing vehicle was prepared by mixing methylcellulose with heated (approximately 60° C) deionized water while stirring and then diluting with water to form a 0.5% solution, which was allowed to cool. Approximately half of the required volume of this solution was added to a calibrated glass mixing container and the specified amount of ginseng was weighed and transferred into the mixing container. The container in which the ginseng was weighed was rinsed at least three times into the mixing container. The contents of the mixing container were diluted to final volume with vehicle, and the formulation was mixed with a vigorous vortex on a stirplate for at least 15 minutes. The dose formulations were prepared three times.	For the first preparation of the 600 mg/mL dose formulation and all preparations of the 500 mg/mL or less dose formulations, the specified weight of ginseng was placed into a calibrated glass mixing container with approximately half the required volume of warm (approximately 30° C) sterile water, stirred with a vigorous vortex for at least 15 minutes on a stirplate, and then diluted to the required total volume with additional warm sterile water. For the 800 and 1,000 mg/mL dose formulations and all but the first 600 mg/mL dose formulation, a calibrated glass mixing bottle was filled with the specified weight of ginseng, a calculated volume of warm sterile water based on the estimated displacement of ginseng, and a large stirbar, and then rolled at least overnight on a jar roller. If excessive foaming occurred, the glass mixing bottle was placed in a 30° C water bath. If the final volume was less than the required volume, additional warm sterile water was added and the final contents were gently shaken to mix. The dose formulations were prepared approximately monthly.	For the initial 125, 250, and 500 mg/mL dose formulations, approximately half of the required volume of warmed (approximately 30° C) sterile water was placed in a calibrated Nalgene® carboy into which the appropriate amount of ginseng had been weighed, and the contents of the carboy were vigorously mixed for 10 minutes with an overhead stirrer. A specified volume was dispensed from the tap of the carboy into a beaker and poured back into the carboy. The beaker was rinsed with warm sterile water, the contents of the carboy were diluted to the specified final volume, and the contents were vigorously mixed for an additional 5 minutes. Due to excessive foaming, for subsequent preparations of the dose formulations, stirring was slowed, and the sides and bottom of the carboy were scraped with a spatula that was rinsed with a small amount of sterile water into the carboy. For the 1,000 mg/mL dose formulation, the appropriate weight of ginseng was added to the specified volume of warmed (approximately 30° C) sterile water in a calibrated glass mixing bottle containing a large stirbar. After sealing, the bottle was rolled on a jar roller at least overnight; the bottle was intermittently placed in a 30° C water bath to aid dissolution of the test material. The dose formulations were prepared approximately monthly.
Chemical Lot Number		
3021261	302500702	3031978
Maximum Storage Time		
9 days	39 days	44 days
Storage Conditions		
Stored in amber glass bottles with Teflon® lined lids at approximately 5° C.	Stored in sealed Nalgene® plastic bottles at less than or equal to 20° C.	Stored in sealed Nalgene® plastic bottles at less than or equal to 20° C.
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Gavage Studies of Ginseng

Date Prepared	Date Analyzed	Target Concentration[a] (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
October 4, 2002	October 4-5, 2002	12.5	12.70	+2
		25	26.31	+5
		50	52.22	+4
		100	100.5	+1
		200	208.5	+4
		400	433.7	+8
	October 14-16, 2002[c]	25	26.28	+5
		50	52.53	+5
		100	103.6	+4
		200	211.3	+6
		400	448.0	+12
	October 14-16, 2002[d]	12.5	13.21	+6
		25	27.25	+9
		50	52.34	+5
		100	101.8	+2
		200	195.8	–2

[a] The 12.5 and 400 mg/mL dose formulations were used for mice or rats only, respectively.
[b] Results of duplicate analyses. Dosing volume for rats =5 mL/kg; 25 mg/mL=125 mg/kg, 50 mg/mL=250 mg/kg, 100 mg/mL=500 mg/kg, 200 mg/mL=1,000 mg/kg, 400 mg/mL=2,000 mg/kg. Dosing volume for mice=10 mL/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, 200 mg/mL=2,000 mL/kg.
[c] Rat animal room samples
[d] Mouse animal room samples

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Ginseng

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
March 3, 2003	March 5-7, 2003	200	199.4	0
		400	389.6	–3
		600	591.1	–1
		800	750.1	–6
		1,000	947.4	–5
	April 16-18, 2003[b]	200	204.2	+2
		400	411.9	+3
		600	590.6	–2
		800	773.5	–3
		1,000	1,035	+4
March 24, 2003	April 1-4, 2003			
		200	188.5	–6
		400	373.7	–7
		600	576.8	–4
		800	779.4	–3
		1,000	949.1	–5
	May 6-8, 2003[b]	200	204.6	+2
		400	400.7	0
		600	601.0	0
		800	746.4	–7
		1,000	967.9	–3
May 19, 2003	May 22-28, 2003	200	197.2	–1
		400	401.8	+1
		600	610.4	+2
		800	810.7	+1
		800	820.5	+3
		1,000	985.9	–1
		1,000	983.5	–2
	June 19-24, 2003[b]	200	192.0	–4
		400	365.7	–9
		600	599.3	0
		800	794.6	–1
		800	768.7	–4
		1,000	990.3	–1
		1,000	—[c]	—

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Ginseng

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
March 3, 2003	March 5-7, 2003	100	99.39	–1
		200	199.4	0
		300	301.4	0
		400	389.6	–3
		500	513.1	+3
	April 16-18, 2003[b]	100	101.8	+2
		200	206.2	+3
		300	308.0	+3
		400	406.5	+2
		500	514.1	+3
March 24, 2003	April 1-4, 2003	100	91.78	–8
		200	188.5	–6
		300	278.3	–7
		400	373.7	–7
		500	469.9	–6
	May 6-8, 2003[b]	100	96.61	–3
		200	198.5	–1
		300	304.8	+2
		400	406.6	+2
		500	498.5	0
May 19, 2003	May 22-28, 2003	100	102.2	+2
		200	197.2	–1
		300	304.5	+2
		400	401.8	+1
		500	493.1	–1
	June 19-24, 2003[b]	100	100.5	+1
		200	192.0	–4
		300	293.2	–2
		400	388.4	–3
		500	497.8	0

[a] Results of duplicate analyses. Dosing volume for rats =5 mL/kg; 200 mg/mL=1,000 mg/kg, 400 mg/mL=2,000 mg/kg, 600 mg/mL=3,000 mg/kg, 800 mg/mL=4,000 mg/kg, 1,000 mg/mL=5,000 mg/kg. Dosing volume for mice=10 mL/kg; 100 mg/mL=1,000 mg/kg, 200 mg/mL=2,000 mg/kg, 300 mg/mL=3,000 mg/kg, 400 mg/mL=4,000 mg/kg, 500 mg/mL=5,000 mg/kg.
[b] Animal room samples
[c] Not analyzed; this dose formulation was not used after June 6, 2003, due to excessive foaming.

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Ginseng

Date Prepared	Date Analyzed	Target Concentration[a] (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
January 5, 2004	January 16-17, 2004	125	131.7	+5
		250	257.4	+3
		500	514.2	+3
		1,000	1,019	+2
		1,000	1,006	+1
		1,000	993.0	–1
	March 2-4, 2004[c]	250	255.6	+2
		500	501.0	0
January 26, 2004	February 3-4, 2004	1,000	1,014	+1
	March 8-9, 2004[c]	1,000	968.4	–3
	March 2-9, 2004[d]	125	129.5	+4
		250	234.9	–6
		500	499.6	0
March 29, 2004	April 9-14, 2004	125	126.4	+1
		250	258.6	+3
		500	510.1	+2
		1,000	1,030	+3
		1,000	1,005	+1
		1,000	1,028	+3
		1,000	1,029	+3
		1,000	1,024	+2
		1,000	1,020	+2
		1,000	964.3	–4
		1,000	1,014	+1
		1,000	998.9	0
		1,000	938.4	–6
June 14, 2004	June 23-24, 2004	125	128.1	+2
		250	250.2	0
		500	503.8	+1
		1,000	1,057	+6
		1,000	1,064	+6
		1,000	1,042	+4
		1,000	1,041	+4
		1,000	1,032	+3

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Ginseng

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
September 7, 2004	September 15-22, 2004	125	127.5	+2
		250	228.6	–9
		500	531.2	+6
		1,000	995.5	0
		1,000	1,013	+1
		1,000	1,023	+2
		1,000	1,049[e]	+5
		1,000	946.0	–5
	October 27-29, 2004[c]	250	246.3	–1
		500	544.8	+9
		1,000	1,044	+4
	October 27-29, 2004[d]	125	136.1	+9
		250	247.7	–1
		500	540.1	+8
November 29, 2004	December 8-9, 2004	125	128.5	+3
		250	260.8	+4
		500	505.4	+1
		1,000	1,060	+6
		1,000	1,107	+11
		1,000	1,060	+6
		1,000	1,059	+6
		1,000	1,077	+8
February 21, 2005	March 3-5, 2005	125	123.2	–1
		250	245.5	–2
		500	469.3	–6
		1,000	998.7	0
		1,000	993.1	–1
		1,000	999.4	0
		1,000	1,004	0
		1,000	1,002	0
May 16, 2005	May 18-21, 2005	125	124.4	0
		250	243.3	–3
		500	481.7	–4
		1,000	998.8	0
		1,000	1,007	+1
		1,000	977.9	–2
		1,000	1,003	0
		1,000	988.0	–1
	June 29-30, 2005[c]	250	248.4	–1
		500	518.8	+4
		1,000	1,048	+5
	June 29-30, 2005[d]	125	132.3	+6
		250	253.7	+1
		500	506.9	+1

TABLE I6
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Ginseng

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
August 8, 2005	August 16-18, 2005	125	126.7	+1
		250	251.5	+1
		500	501.0	0
		1,000	1,018	+2
		1,000	1,019	+2
		1,000	976.8	–2
		1,000	956.8	–4
		1,000	999.3	0
October 31, 2005	November 8-10, 2005	125	130.6	+4
		250	258.6	+3
		500	470.6	–6
		1,000	955.6	–4
		1,000	1,001	0
		1,000	941.5	–6
		1,000	1,013[e]	+1
		1,000	1,032	+3

[a] The 125 and 1,000 mg/mL dose formulations were used for mice or rats only, respectively
[b] Results of duplicate analyses. Dosing volume for rats=5 mL/kg; 250 mg/mL=1,250 mg/kg, 500 mg/mL=2,500 mg/kg, 1,000 mg/mL=5,000 mg/kg. Dosing volume for mice=10 mL/kg; 125 mg/mL=1,250 mg/kg, 250 mg/mL= 2,500 mg/kg, 500 mg/mL=5,000 mg/kg.
[c] Rat animal room samples
[d] Mouse animal room samples
[e] Result of triplicate analyses

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

TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

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

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

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

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

[a] Per kg of finished product

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.7 ± 0.67	13.7 – 16.3	25
Crude fat (% by weight)	8.2 ± 0.41	7.4 – 9.3	25
Crude fiber (% by weight)	9.2 ± 0.49	8.2 – 9.9	25
Ash (% by weight)	4.9 ± 0.25	4.4 – 5.4	25
Amino Acids (% of total diet)			
Arginine	0.770 ± 0.070	0.670 – 0.970	18
Cystine	0.225 ± 0.023	0.150 – 0.250	18
Glycine	0.706 ± 0.043	0.620 – 0.800	18
Histidine	0.362 ± 0.082	0.310 – 0.680	18
Isoleucine	0.542 ± 0.046	0.430 – 0.660	18
Leucine	1.087 ± 0.066	0.960 – 1.240	18
Lysine	0.712 ± 0.118	0.310 – 0.840	18
Methionine	0.407 ± 0.051	0.260 – 0.490	18
Phenylalanine	0.626 ± 0.043	0.540 – 0.720	18
Threonine	0.500 ± 0.046	0.430 – 0.610	18
Tryptophan	0.142 ± 0.024	0.110 – 0.200	18
Tyrosine	0.388 ± 0.058	0.280 – 0.540	18
Valine	0.667 ± 0.045	0.550 – 0.730	18
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.243	3.49 – 4.54	18
Linolenic	0.30 ± 0.035	0.21 – 0.35	18
Vitamins			
Vitamin A (IU/kg)	4,370 ± 81	3,150 – 6,160	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm)[b]	7.9 ± 1.20	6.3 – 10.5	25
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B_{12} (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	0.968 ± 0.051	0.884 – 1.080	25
Phosphorus (%)	0.571 ± 0.027	0.525 – 0.623	25
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1,380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

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

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

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.22 ± 0.065	0.14 – 0.39	25
Cadmium (ppm)	0.06 ± 0.020	0.04 – 0.10	25
Lead (ppm)	0.09 ± 0.020	0.06 – 0.13	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.25 ± 0.087	0.18 – 0.49	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	12.5 ± 5.05	4.8 – 24.4	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	10 ± 0.0	10.0	25
Coliform (MPN/g)	3.0 ± 0.0	3.0	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.9 ± 1.90	2.3 – 9.9	25
N-Nitrosodimethylamine (ppb)[e]	2.8 ± 1.41	1.1 – 6.3	25
N-Nitrosopyrrolidine (ppb)[e]	2.1 ± 0.80	1.1 – 4.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.118 ± 0.138	0.020 – 0.416	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.25 ± 0.204	0.020 – 0.997	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride

[b] For values less than the limit of detection, the detection limit is given as the mean.

[c] Sources of contamination: alfalfa, grains, and fish meal

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

[e] All values were corrected for percent recovery.

APPENDIX K
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

For the 3-month study, serum samples were collected from five male and five female sentinel rats and mice at 1 month and at the end of the studies; for the 2-year studies, samples were collected from five male and five female sentinel rats and mice at 1, 6, 12, and 18 months and from 5,000 mg/kg male and female rats and mice at the end of the studies. Fecal samples were obtained from male and female sentinel mice at 18 months for testing for *Helicobactor hepaticus sp*. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

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

Method and Test	Time of Collection
MICE	
3-Month Study	
ELISA	
Ectromelia virus	1 month and study termination
EDIM (epizootic diarrhea of infant mice)	1 month and study termination
GDVII (mouse encephalomyelitis virus)	1 month and study termination
LCM (lymphocytic choriomeningitis virus)	1 month and study termination
MHV (mouse hepatitis virus)	1 month and study termination
Mouse adenoma virus-FL	1 month and study termination
PVM	1 month and study termination
Reovirus 3	1 month and study termination
Sendai	1 month and study termination
Immunofluorescence Assay	
Parvovirus	1 month and study termination
2-Year Study	
ELISA	
Ectromelia virus	1, 6, 12, and 18 months, study termination
EDIM	1, 6, 12, and 18 months, study termination
GDVII	1, 6, 12, and 18 months, study termination
LCM	1, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	1, 6, 12, and 18 months, study termination
MHV	1, 6, 12, and 18 months, study termination
MMV VP2 (Mouse minute virus, viral protein 2)	6, 12, and 18 months, study termination
MPV VP2 (Mouse parvovirus, viral protein 2)	6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	1, 6, 12, and 18 months, study termination
Reovirus 3	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Ectromelia virus	6 months and 18 months
EDIM	Study termination
GDVII	18 months
Mouse adenoma virus-1	12 months
Mouse adenoma virus-FL	18 months
MCMV (mouse cytomegalovirus)	Study termination
Parvovirus	1 month
PVM	18 months
Polymerase Chain Reaction	
Helicobacter species	18 months
Western Blot	
PVM	Study termination

RESULTS

All test results were negative.

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