NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF GOLDENSEAL ROOT POWDER (*HYDRASTIS CANADENSIS*)

IN F344/N RATS AND B6C3F1 MICE

(FEED STUDIES)

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

August 2010

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

FOREWORD

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

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

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.

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*) or in hardcopy upon request from the NTP Central Data Management group at *cdm@niehs.nih.gov* or (919) 541-3419.

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SUMMARY

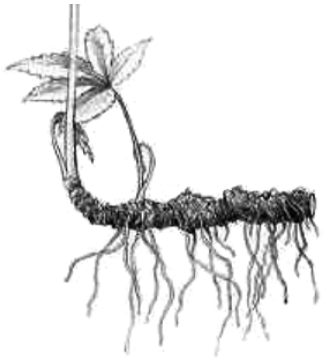
Background
Goldenseal is a plant that has been used in herbal medicine for the treatment of gastrointestinal and urinary disorders and skin, mouth, and eye infections. Extracts of goldenseal root are dried to make teas or liquid extracts or combined with echinacea in tablets. We studied the effects of goldenseal root powder given to rats and mice in the feed to identify potential toxic or cancer-related hazards.

Methods
We gave feed containing 3,000, 9,000, or 25,000 parts per million (ppm) of goldenseal root powder to groups of 50 male and female rats and mice for two years. Similar groups of animals were given feed 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
Survival of all exposed groups of animals was similar to their controls. The rates of cancer (hepatocellular adenoma) of the liver was markedly increased in male and female rats receiving 25,000 ppm goldenseal root powder, and male mice receiving goldenseal root powder had increased rates of liver hepatoblastomas and multiple hepatocellular adenomas.

Conclusions
We conclude that goldenseal root powder caused cancer in the liver of male and female rats and male mice. There was no effect of goldenseal root powder on female mice.

ABSTRACT



GOLDENSEAL ROOT POWDER (*HYDRASTIS CANADENSIS*)

Synonyms/Common Names/Related Substances: Berberine; berberine bisulfate; curcuma; eye balm; eye root; golden root; goldensiegel; goldsiegel; ground raspberry; guldsegl; hydrastidis rhizoma; hydrophyllum; Indian dye, Indian paint, Indian plant, Indian turmeric; jaundice root; kanadische gelbwurzel; kurkuma; Ohio curcuma; orange root; tumeric root; warnera; wild curcuma; wild turmeric; yellow eye; yellow Indian plant; yellow paint; yellow paint root; yellow puccoon; yellow root; yellow seal; yellow wort.

Note: Goldenseal is sometimes referred to as Indian turmeric or curcuma, but should not be confused with turmeric (Curcuma longa Linn.).

Goldenseal root powder is used in folk medicine for the treatment of gastrointestinal disturbances, urinary disorders, hemorrhage, skin, mouth, and eye infections, and inflammation. The major alkaloids in goldenseal are berberine, hydrastine, and canadine. Goldenseal root powder was nominated for study by the National Institute of Environmental Health Sciences based on the potential for human exposure and the lack of carcinogenicity data, and because it is one of the most widely used herbs in the United States. Male and female F344/N rats and B6C3F1 mice were exposed to ground goldenseal root powder in feed for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium, Escherichia coli,* mouse bone marrow cells, and mouse peripheral blood erythrocytes.

2-Week Study in Rats

Groups of five male and five female rats were fed diets containing 0, 1,560, 3,121, 6,250, 12,500, 25,000, or 50,000 ppm goldenseal root powder (equivalent to average daily doses of approximately 155, 315, 630, 1,190, 2,465, and 4,815 mg goldenseal root powder/kg body weight for males and 150, 290, 640, 1,240, 2,370, and 4,870 mg/kg for females) for 15 days. All rats survived to the end of the study. Mean body weights and feed consumption of all exposed groups of males and females were similar to those of the control groups throughout the study. Liver weights of males exposed to 6,250 ppm or greater and females exposed to 12,500 ppm or greater were significantly greater than those of the controls. Minimal to moderate hepatocellular hypertrophy occurred in three males and all females exposed to 25,000 ppm and in all 50,000 ppm males and females.

2-Week Study in Mice

Groups of five male and five female mice were fed diets containing 0, 1,560, 3,121, 6,250, 12,500, 25,000, or 50,000 ppm goldenseal root powder (equivalent to average daily doses of approximately 380, 840, 1,760, 3,435, 6,700, and 15,170 mg/kg body weight for males and 330,

670, 1,240, 2,375, 4,760, and 8,475 mg/kg for females) for 15 days. All mice survived to the end of the study. Mean body weights and feed consumption of all exposed groups of males and females were similar to those of the control groups throughout the study. Significant increases in liver weights occurred in males exposed to 25,000 and 50,000 ppm and in females exposed to 50,000 ppm. Absolute and relative thymus weights of 12,500 and 50,000 ppm males were significantly decreased. Minimal hypertrophy of centrilobular hepatocytes occurred in all males and females exposed to 50,000 ppm.

3-Month Study in Rats

Groups of 10 male and 10 female rats were fed diets containing 0, 3,121, 6,250, 12,500, 25,000, or 50,000 ppm goldenseal root powder (equivalent to average daily doses of approximately 255, 500, 1,000, 2,020, and 4,060 mg/kg for males and 260, 500, 1,030, 2,070, and 4,100 mg/kg for females) for 14 weeks. Additional groups of 10 male and 10 female clinical pathology study rats were given the same concentrations for 23 days. All rats survived to the end of the study. None of the body weights or mean body weight gains were significantly different from those of the controls. Feed consumption by exposed groups was generally similar to that by controls throughout the study. Liver weights were significantly increased in males exposed to 6,250 ppm or greater and in all exposed groups of females. The incidences of hepatocyte hypertrophy were significantly increased in the liver of males and females exposed to 12,500 ppm or greater; cytoplasmic vacuolization of hepatocytes occurred in all exposed males.

3-Month Study in Mice

Groups of 10 male and 10 female mice were fed diets containing 0, 3,121, 6,250, 12,500, 25,000, or 50,000 ppm goldenseal root powder (equivalent to average daily doses of approximately 680, 1,360, 2,260, 5,370, and 10,550 mg/kg for males and 590, 1,250, 2,345, 4,790, and 10,740 mg/kg for females) for 14 weeks. All mice survived to the end of the study. Mean body weights of males exposed to 50,000 ppm and females exposed to 25,000 or 50,000 ppm were significantly less than those of the controls. Feed consumption by 3,121, 6,250, 12,500, 25,000, and 50,000 ppm males was similar to that by controls. Liver weights were significantly increased in males exposed to 12,500 ppm or greater and in females exposed to 25,000 or 50,000 ppm. The left epididymal weight in male mice was significantly decreased relative to controls. The incidences of hepatocyte hypertrophy were significantly increased in males and females exposed to 12,500 ppm or greater.

2-Year Study in Rats

Groups of 50 male and 50 female rats were fed diets containing 0, 3,000, 9,000, or 25,000 ppm goldenseal root powder (equivalent to average daily doses of approximately 135, 400, and 1,175 mg/kg for males and 150, 470, and 1,340 mg/kg for females) for 105 to 106 weeks**.** Survival of 9,000 ppm females was significantly greater than that of the controls. Mean body weights of females exposed to 9,000 ppm were 6% less than those of the controls after week 37, and those of 25,000 ppm females were 6% less than those of the controls after week 8. Feed consumption by exposed groups of males and females was generally similar to that by the controls throughout the study.

The incidences of hepatocellular adenoma were significantly increased in males and females exposed to 25,000 ppm, and the incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in 25,000 ppm males. All exposed groups of males and females had significantly increased incidences of hepatocyte hypertrophy. The incidences of hepatocyte degeneration were significantly increased in all exposed groups of males and in 9,000 and 25,000 ppm females. The incidences of eosinophilic focus were significantly increased in 9,000 and 25,000 ppm males and all exposed groups of females.

The incidences of cardiomyopathy were significantly decreased in all exposed groups of males and in 25,000 ppm females.

2-Year Study in Mice

Groups of 50 male and 50 female mice were fed diets containing 0, 3,000, 9,000, or 25,000 ppm goldenseal root powder (equivalent to average daily doses of approximately 375, 1,120, and 3,275 mg/kg for males and 330, 1,000, and 2,875 mg/kg for females) for 105 to 106 weeks. Survival of 9,000 ppm females was significantly less than that of the controls. Mean body weights of females exposed to 25,000 ppm were 3% to 9% less than those of the controls after week 13, 6% less for weeks 14 to 52, and 5% less for weeks 53 to 101. Feed consumption by exposed groups of males and females

was generally similar to that of the controls throughout the study.

The incidences of hepatocellular adenoma occurred with a positive trend in males, and the incidences of multiple hepatocellular adenoma were significantly increased in 9,000 and 25,000 ppm males. The incidences of hepatoblastoma occurred with a positive trend in males with a marginal increase in the 25,000 ppm group. Significantly increased incidences of eosinophilic focus or mixed cell focus occurred in all exposed groups of males.

Genetic Toxicology

Goldenseal root powder was not mutagenic in *Salmonella typhimurium* or *Escherichia coli* tester strains, with or without liver S9 metabolic activation enzymes. In addition, no increases in the frequencies of micronucleated erythrocytes were observed in peripheral blood samples from mice exposed to goldenseal root powder in feed for 3 months.

Berberine chloride was also tested for mutagenicity in standard screening assays. No mutagenicity was observed in several tester strains of *Salmonella typhimurium*, with or without rat or hamster liver S9 metabolic activation enzymes. In an acute exposure assay, no increase in the frequency of micronucleated polychromatic erythrocytes was seen in bone marrow of male mice administered three intraperitoneal injections of berberine chloride at 24-hour intervals.

Conclusions

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity** of goldenseal root powder in male F344/N rats based on the increased incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined). There was *clear evidence of carcinogenic activity* of goldenseal root powder in female F344/N rats based on the increased incidence of hepatocellular adenoma. There was *some evidence of carcinogenic activity* of goldenseal root powder in male B6C3F1 mice based on the increased incidences of hepatoblastoma and multiple hepatocellular adenoma. There was *no evidence of carcinogenic activity* of goldenseal root powder in female B6C3F1 mice exposed to 3,000, 9,000, or 25,000 ppm goldenseal root powder in feed for 2 years.

Administration of goldenseal root powder resulted in increased incidences of nonneoplastic lesions in the liver of male and female rats and male mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Goldenseal Root Powder

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations in feed	0, 3,000, 9,000, or 25,000 ppm	0, 3,000, 9,000, or 25,000 ppm	0, 3,000, 9,000, or 25,000 ppm	0, 3,000, 9,000, or 25,000 ppm
Body weights	Exposed groups similar to the control group	9,000 ppm group 6% less than the control group after week 37; 25,000 ppm group 6% less than the control group after week 8	Exposed groups similar to the control group	25,000 ppm group 3% to 9% less than the control group after week 13
Survival rates	33/50, 29/50, 30/50, 25/50	30/50, 36/50, 42/50, 36/50	43/50, 38/50, 45/50, 45/50	45/50, 44/50, 36/50, 43/50
Nonneoplastic effects	Liver: hepatocyte, hypertrophy (0/50, 19/50, 31/50, 27/50); hepatocyte, degeneration (0/50, 22/50, 30/50, 19/50); eosinophilic focus (4/50, 5/50, 25/50, 28/50)	Liver: hepatocyte, hypertrophy (2/50, 10/50, 27/50, 38/50); hepatocyte, degeneration (1/50, 2/50, 12/50, 24/50); eosinophilic focus (2/50, 24/50, 29/50, 22/50)	Liver: eosinophilic focus (7/50, 14/50, 14/50, 24/50)	None
Neoplastic effects	Liver: hepatocellular adenoma (1/50, 1/50, 2/50, 10/50); hepatocellular adenoma or carcinoma (1/50, 1/50, 2/50, 11/50)	Liver: hepatocellular adenoma (0/50, 0/50, 1/50, 8/50)	Liver: hepatoblastoma (1/50, 2/50, 1/50, 6/50); multiple hepatocellular adenoma (3/50, 5/50, 11/50, 18/50)	None
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Some evidence	No evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:				
Goldenseal root powder		Negative in strains TA98 and TA100 with and without S9 and in *Escherichia coli* WPM *uvrA*/pKM101 with and without S9		
Berberine chloride		Negative in strains TA97, TA98, TA100 and TA1535 with and without S9		
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*				
Goldenseal root powder		Negative		
Mouse bone marrow *in vivo:*				
Berberine chloride		Negative		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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 in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on goldenseal root powder on February 25, 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 February 25, 2009, the draft Technical Report on the toxicology and carcinogenesis studies of goldenseal root powder (Hydrastis canadensis) received public review by the National Toxicology Program's Board of Scientific Counselors Technical Report Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. June Dunnick, NIEHS, introduced the studies of goldenseal root powder by reviewing its use as a natural herbal product, the nomination of goldenseal root powder for study by the NTP, and the major plant alkaloids found in goldenseal and by describing the design of the short- and long-term studies and the effects of goldenseal root powder on survival, body weight, and liver lesions in rats and mice. The proposed conclusions were:

> Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity* of goldenseal root powder in male F344/N rats based on the increased incidences of hepatocellular adenomas and hepatocellular adenomas or carcinomas (combined). There was *clear evidence of carcinogenic activity* of goldenseal root powder in female F344/N rats based on the increased incidence of hepatocellular adenoma. There was *some evidence of carcinogenic activity* of goldenseal root powder in male B6C3F1 mice based on the increased incidences of hepatoblastoma and multiple hepatocellular adenoma. There was *no evidence of carcinogenic activity* of goldenseal root powder in female B6C3F1 mice exposed to 3,000, 9,000, or 25,000 ppm goldenseal root powder in feed for 2 years.
>
> Administration of goldenseal root powder resulted in increased incidences of nonneoplastic lesions in the liver of male and female rats and male mice.

Dr. Raymond Novak noted that two written comments were submitted on behalf of the American Herbal Products Association. Mr. Michael McGuffin, representing the American Herbal Products Association, spoke to clarify that goldenseal was not likely to be consumed for long periods of time by humans and that the highest daily dose used in the study was 1.5 times higher than would be consumed by humans daily.

Dr. Tracie Bunton, the first primary reviewer, said the report clearly described the progression of the development of the liver lesions over time, and agreed with the conclusions.

Dr. Justin Teeguarden, the second primary reviewer, said the report was written clearly and the dose selection appeared appropriate. He asked if the conclusions could present comparison with expected human dosages and also if trends for decreases in tumor incidence would be noted.

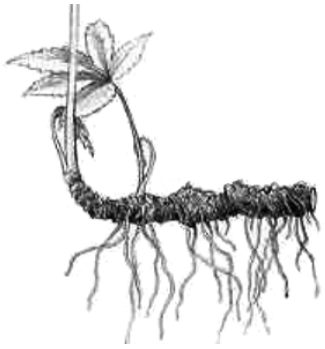
Dr. James Sherley, the third primary reviewer, also agreed with the conclusions.

Dr. Dunnick noted that the actual human intake levels are largely unknown, and also that this NTP report is a hazard identification rather than a risk assessment document. Dr. John Bucher, NIEHS, explained that in general, the NTP avoids including dose levels in conclusions where carcinogenic effects were seen, as often the total dose range that elicits a carcinogenic response is not known. In cases where there is no evidence of carcinogenicity, the doses are included in the conclusion statements as this is important information.

Dr. Michael Pino asked for clarification on combining the incidences of adenoma and carcinoma in the conclusion statement, when only one carcinoma was observed. Dr. David Malarkey, NIEHS, said that adenomas and carcinomas were considered part of a continuum of progressive lesions and that the number of adenomas was driving the call. Dr. Michelle Hooth, NIEHS, noted that in six concurrent studies no liver carcinomas had been seen in 300 control male rats.

Dr. Kenneth Portier moved and Dr. David Eastmond seconded that the conclusion be accepted as written. The motion was approved unanimously with 8 yes votes, 0 no votes, and 0 abstentions.

INTRODUCTION



GOLDENSEAL ROOT POWDER (*HYDRASTIS CANADENSIS*)

Synonyms/Common Names/Related Substances: Berberine; berberine bisulfate; curcuma; eye balm; eye root; golden root; goldensiegel; goldsiegel; ground raspberry; guldsegl; hydrastidis rhizoma; hydrophyllum; Indian dye, Indian paint, Indian plant, Indian turmeric; jaundice root; kanadische gelbwurzel; kurkuma; Ohio curcuma; orange root; tumeric root; warnera; wild curcuma; wild turmeric; yellow eye; yellow Indian plant; yellow paint; yellow paint root; yellow puccoon; yellow root; yellow seal; yellow wort.

Note: Goldenseal is sometimes referred to as Indian turmeric or curcuma, but should not be confused with turmeric (Curcuma longa Linn.).

Chemical and Physical Properties

Goldenseal (also known as *Hydrastis canadensis*) is a plant that has been used in herbal medicine (NCCAM, 2007). The chemical profile of the goldenseal root powder used in the current NTP studies is similar to other goldenseal chemical profiles reported in the literature with hydrastine, berberine, canadine, and other minor isoquinoline alkaloids present (Hamon, 1990; Abourashed and Khan, 2001; Brown and Roman, 2008). Standardization of goldenseal is usually based on the content of hydrastine and berberine with not less than 2% hydrastine and 2.5% berberine in powdered dried roots and rhizomes (Li and Fitzloff, 2002). The alkaloid content in goldenseal may vary from batch to batch (Edwards and Draper, 2003). A thorough evaluation of several lots of goldenseal root powder has been published (Weber *et al*., 2003a,b) which shows that most of the content is complex plant material and purity of these products requires both analysis of the product to confirm the isoquinoline alkaloids as above and also to confirm the absence of other isoquinoline alkaloids characteristic of common adulterants such as coptis root, Oregon grape root, and others.

The major alkaloids in the goldenseal root powder used in the studies presented in this Technical Report included berberine, hydrastine, and canadine (Figure 1) and other minor alkaloids characteristic of goldenseal (MRI, 2003; NTP 2003a,b) as determined by the method of Weber *et al*. (2003b). The major alkaloid content for goldenseal used in the 2-week and 3-month studies was 3.45% berberine, 3.02% hydrastine, and 0.08% canadine by weight. The major alkaloid content for goldenseal used in the 2-year study was 3.89% berberine, 2.8% hydrastine and 0.17% canadine. No alkaloids characteristic of common adulterants were found in either lot of the goldenseal root powder used in the 2-week, 3-month, or 2-year studies. Nutritional screens were also performed for both lots and mineral content, pesticide content, and microbiological content were all deemed acceptable for use in these studies.

Production, Use, and Human Exposure

Goldenseal

Goldenseal (*Hydrastis canadensis*) is a member of the plant family *Ranunculaceae* (*Merck*, 1996; NMCD, 2008) with a history of use in folk medicine for the occasional treatment of gastrointestinal disturbances; urinary disorders; hemorrhage; skin, mouth, and eye infections; and inflammation (Hamon, 1990; Abourashed and Khan, 2001; NCCAM, 2007; Brown and Roman, 2008). Goldenseal is applied to wounds and canker sores and is used as a mouthwash for sore gums, mouth, and throat. Goldenseal has been used as an antispasmodic and laxative and to treat anorexia, gastritis, peptic ulcer, colitis, liver conditions, inflammation, and hemorrhage. Claims in the lay press note that goldenseal can treat menstrual disorders, minor sciatica, and rheumatic and muscular pain, although there have been few controlled clinical trials to establish efficacy for any of these therapeutic uses (NCCAM, 2007).

Goldenseal in combination with echinacea was one of the top 10 herbal supplements sold in the United States in 1999 with estimated sales of $70 million in 1998 (Blumenthal, 1999). Children in the U. S. taking nonvitamin/nonmineral natural products often take goldenseal (Barnes *et al.*, 2008).

The underground stems or roots of the goldenseal plant are dried and used to make teas, liquid extracts, and solid extracts that may be made into tablets and capsules. Goldenseal is often combined with echinacea in tablets sold in health food stores (NCCAM, 2007).

A typical goldenseal dose is taken orally three times a day (0.5 to 1 gram goldenseal per day) in a tablet or in a capsule, or as a liquid/fluid extract, decoction, or as a tincture (NSD, 2007a). For infectious diarrhea, 100 to 200 mg of berberine hydrochloride may be taken by mouth four times daily or as a single daily dose of 400 mg. Berberine sulfate is often used as well, and the hydrochloride and sulfate forms are generally thought to have equivalent effects.

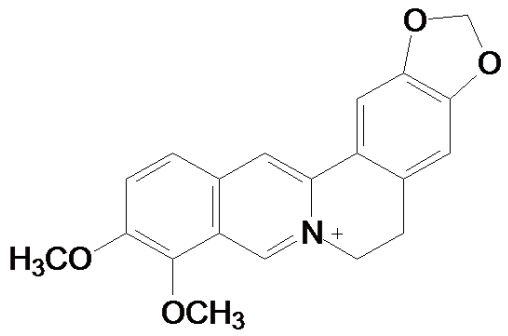
Goldenseal Alkaloid: Berberine

Berberine, a widely studied goldenseal alkaloid, is usually present in plants as a sulfate (HSDB, 2008). It is found in the rhizomes of Chinese Goldthread (*C. chinensis* Franch., 40,000 to 90,000 ppm), generic Goldthread (*Coptis* spp, 40,000 to 90,000 ppm), and Huang-Lia (*C. japonica*, 40,000 to 70,000 ppm); in the roots of goldenseal (5,000 to 60,000 ppm), in the bark of Huang Po (*Phellodendron amurense* Rupr., 8,300 to 10,000 ppm); and in the plant parts of Barberry (*Berberis vulgaris* L., 10,000 to 30,000 ppm) and Prickly Poppy (*Argemone mexicana* L., 410 ppm) (Beckstrom-Sternberg and Duke, 1997a). Berberine is also a constituent (concentrations not given) of *B. aristata, B. lamberti, B. asiatica, B. heterobotrus, B. crataegina, B. cretica, B. thunbergii, B. kawakamii, B. mingetsensis, B. morrisonensis, B. francesciferdinandi, B. koreana, B. iliensis, B. guimpeli, B. lycium, B. peteolaris, and B. amurensis var* (Ikram, 1975).

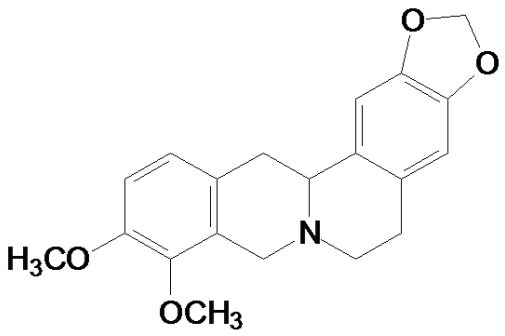
Berberine can be produced from cultures of *Coptis japonica* cells (Fujita and Tabata, 1987) and *Thalictrum rugosum* cells (Kim *et al*., 1990). Adding gibberellic acid to *C. japonica* cell cultures (Fujita, 1988) or cupric sulfate to *T. rugosum* cell cultures (Kim *et al*., 1991) increases the yield. Also, producing *C. japonica* and *T. rugosum* at high cell density is essential for maximizing production yields (Piehl *et al*., 1988; Kim *et al*., 1990).

Berberine is reported to have activity against a variety of infectious agents. It is reported to have antimicrobial activity against *Staphylococcus aureus* in cell culture (Stermitz *et al.*, 2000); antifungal activity against *Candida* species (Volleková *et al.*, 2003); antimalarial activity against *Plasmodium falciparum* (Tran *et al.*, 2003); and activity against cholera (Kulkarni *et al.*, 1972), amoebiasis (Sabir *et al.*, 1978), and *Leishmania* and *Plasmodium* in golden hamsters (Vennerstrom *et al.*, 1990). Berberine has also been reported to have anti-inflammatory (Stermitz *et al.*, 2000; Kuo *et al.*, 2004), cytostatic (Jantová *et al.*, 2006; Grycová *et al.*, 2007; Maiti and Kumar, 2007; Serafim *et al.*, 2008), antiproliferative (Müller *et al.*, 1995; Letašiová *et al.*, 2005), and antioxidative (Shirwaikar *et al.*, 2006) activities.

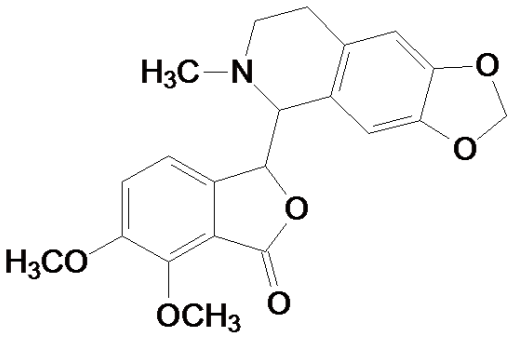
When berberine was given at 5 mg/kg body weight per day for 6 days to patients with giardiasis, it was reported to help clear the infection (Choudhry *et al.*, 1972). Berberine administered as a single dose of 400 mg to patients with acute diarrhea due to *Escherichia coli* or *Vibrio cholerae* was reported to help reduce mean stool volumes (Rabbani *et al.*, 1987). Although these therapeutic effects have been attributed to berberine, the chemical is not approved for use as a drug (NSD, 2007b).



Berberine



Canadine



Hydrastine

FIGURE 1
Major Alkaloid Constituents of Goldenseal

Berberine is used as a fluorescent stain in medical research (Kim *et al.*, 1990) to stain cells (Borodina *et al.*, 1979), chromosomes (Ridler and Jennings, 1983), and energized mitochondria (Mikeš and Dadák, 1983; Mikeš and Yaguzhinskij, 1985).

Goldenseal Alkaloid: Hydrastine

Hydrastine is claimed to be an abortifacient, antibiotic, antiuterotic, antivaginitic, bactericide, central nervous system depressant, choleretic, convulsant, hemostat, hypertensive, hypotensive, pesticide, sedative, uterotonic, and vasoconstrictor (Beckstrom-Sternberg and Duke, 1997b), but these claims have not been investigated in clinical trials.

Hydrastine can be produced from berberine (Moniot and Shamma, 1976). The first step in the synthesis involves a ferricyanide oxidation of berberine to yield oxybisberberine. Treatment with methanolic hydrogen chloride yields 8-methoxyberberinephenolbetaine, which after hydration yields the hydrochloride salt of dehydronorhydrastine methyl ester. *N*-alkylation then gives dehydrohydrastine methyl ester, and direct sodium borohydride reduction gives a 90% yield of a 1:2 mixture of (±)-α-hydrastine and (±)-β-hydrastine.

Regulatory Status

Goldenseal is considered a dietary supplement as specified by the Dietary Supplement Health and Education Act (DSHEA) of 1994, and the DSHEA places dietary supplements in a special category under the general umbrella of "foods" (USFDA, 1994). For dietary supplements on the market prior to October 15, 1994, the DSHEA requires no proof of safety for them to remain on the market. The labeling requirements for 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).

Absorption, Distribution, Metabolism, and Excretion

Although goldenseal contains non-alkaloids (Weber *et al*., 2003a), the plant's alkaloid constituents are of primary concern for pharmacological effects. Studies of the metabolism and pharmacokinetics of these alkaloids in animals and humans appear to be limited to berberine. These and other studies demonstrating effects of berberine, hydrastine, and extracts of goldenseal on the activity of specific metabolizing enzymes and efflux transporters are described below.

Experimental Animals

Goldenseal Alkaloids

Berberine was absorbed from the gastrointestinal tract of rats following oral administration (Zuo *et al*., 2006; Deng *et al*., 2008; Qiu *et al*., 2008). The extent of absorption was not determined in these studies; however Zuo *et al*. (2006) estimated that 34% of 0.02 mM berberine was absorbed in 1 hour from an *in situ* loop of small intestine in a male Wistar rat. Berberine reached a peak concentration at 2 hours in the plasma of male Wistar rats receiving a single oral dose of 40 mg/kg. The parent chemical was rapidly eliminated from plasma, but was still detected at low levels 48 hours after dosing. The terminal elimination half-life for berberine in plasma was approximately 5.6 hours for male Sprague-Dawley rats gavaged with a preparation of *C. rhizoma* and *Evodia fructus* (Deng *et al*., 2008). Multiple peak concentrations for berberine in plasma were detected over time indicating redistribution from tissues to plasma and/or enterohepatic circulation. A similar elimination half-life for berberine (mean of 5.3 hours) was observed in the blood of rabbits administered 2 mg/kg berberine by intravenous injection (Chen and Chang, 1995). The mean distribution half-life in these animals was 0.04 hours. Berberine appears to be actively transported in the liver. Reduced amounts of the chemical were excreted in the bile of male Sprague-Dawley rats administered intravenous injections of 10 to 20 mg/kg following inhibition of P-glycoprotein (P-gp) by cyclosporin A (Tsai and Tsai, 2004).

Berberine appears to be rapidly metabolized by the liver and excreted in the urine. In rabbits, approximately 5% of an intravenous dose was excreted unchanged in the urine within 48 hours of dosing (Chen and Chang, 1995). The fate of the remainder of the dose was not determined. The bile of intravenously injected rats contained berberine and two metabolites, one derived from demethylation at either the C9 or C10 position and the other being the glucuronide conjugate of the initial O-demethylation reaction (Tsai and Tsai, 2004). Berberine metabolism was decreased following inhibition of cytochrome P450 with SKF-525A. Zuo

et al. (2006) identified major metabolites of berberine in plasma, bile, and liver extracts of rats as berberrubine, thalifendine, demethyleneberberine, and jatrorrhizine (Figure 2) and their respective glucuronide conjugates. Zuo *et al*. (2006) also indicated that these metabolites, as well as parent berberine were present in the urine. Comparative data from germ-free rats (treated with antibiotics) indicated that in the conventional rats, the conjugates were hydrolyzed by gut microflora, resulting in enterohepatic circulation of the aglycones. Qiu *et al*. (2008) identified conjugates in urine of Wistar rats receiving 100 mg/kg berberine by gavage as berberrubine-9-*O*-ß-D-glucuronide, jatrorrhizine-3-*O*-ß-D-glucuronide, demethyleneberberine-2,3-di-*O*-ß-D-glucuronide, demethyleneberberine-2-*O*-sulfate, and 3,10-demethylpalmatine-10-*O*-sulfate. Free thalifendine was also detected in rat urine. The authors did not indicate if berberine was present in the urine.

Humans

As in rats, evidence indicates that berberine is absorbed from the gastrointestinal tract of humans (extent unknown) and is metabolized following absorption. A minor amount of berberine was excreted in the urine of four male volunteers receiving single oral doses of 100 mg berberine chloride (Miyazaki *et al*., 1978). The maximum concentration of berberine in the plasma of 20 male volunteers receiving 400 mg by single oral administration was 0.4 ng/mL, indicating low bioavailability of the parent chemical (Hua *et al*., 2007). The peak concentration time and half-life of berberine in plasma were calculated as 10 and 29 hours, respectively. In a study conducted by Pan *et al*. (2002), three berberine-derived metabolites, as well as a small amount of berberine, were isolated and identified in the urine of five volunteers following oral administration of berberine chloride (900 mg per day for three days). The metabolites were identified as sulfate conjugates, specifically demethyleneberberine-2-*O*-sulfate (the major metabolite), jatrorrhizine-3-*O*-sulfate, and thalifendine-10-*O*-sulfate. Qiu *et al*. (2008) confirmed the presence of the first two sulfate conjugates and identified one other sulfate conjugate, 3,10-demethylpalmatine-10-*O*-sulfate, and four glucuronide conjugates, berberrubine-9-*O*-ß-D-glucuronide, columbamine-2-*O*-ß-D-glucuronide, jatrorrhizine-3-*O*-ß-D-glucuronide, and thalifendine-10-*O*-ß-D-glucuronide in the urine of humans receiving multiple oral doses of 300 mg berberine chloride.

Data from the Pan *et al*. (2002), Zuo *et al*. (2006), and Qiu *et al*. (2008) studies indicate that metabolites of berberine are qualitatively similar in rats and humans. Common metabolites are berberrubine and thalifendine, products of *O*-demethylation at the C9 and C10 positions, respectively, demethyleneberberine, a product of methylenedioxy ring opening and catechol formation, jatrorrhizine (methylation of the catechol), and 3,10-demethylpalmatine, a product of multiple pathways (Figure 2). The major species difference appears to be preferential conjugation of berberine-derived metabolites with sulfate in humans and glucuronic acid in rats.

Results of studies investigating the effects of goldenseal or its alkaloid constituents on human cytochrome P450 activity and eflux transporters suggest that the use of the herb as a dietary supplement could interfere with metabolism and active transport of some pharmaceutical drugs. Aqueous (8% berberine; 4.5% hydrastine) or ethanolic (16.6% berberine; 12.3% hydrastine) extracts of goldenseal or the two individual alkaloids were incubated with human hepatic microsomes (final assay concentrations were 1 or 20 µM alkaloids) and the effect on specific cytochrome P450 enzymes was determined (Etheridge *et al*., 2007). At the high concentration, the goldenseal extracts or berberine inhibited CYP2D6 activity by up to 78%; whereas the goldenseal extracts or hydrastine inhibited CYP3A4 activity by up to 77%. Modest inhibitory effects were observed on CYP2E1 by berberine and on CYP1A2 by hydrastine at the high concentration. Raner *et al*. (2007) demonstrated strong inhibition (55%) of CYP2E1 activity in human hepatic microsomes by goldenseal extract and alkaloid constituents, particularly hydrastine (64% inhibition). CYP2C9, CYP2D6, and CYP3A4 activities were inhibited by goldenseal extract in human hepatic microsomes in work conducted by Chatterjee and Franklin (2003). Further, CYP3A4 gene expression was shown to be affected in Caco-2 cells following incubation with goldenseal extract (Budzinski *et al*., 2007). In human *in vivo* experiments, CYP2D6 and CYP3A4/5 activity was significantly inhibited in women receiving goldenseal extract for 28 days and CYP2D6 activity was inhibited up to 50% in women receiving goldenseal extract for 14 days (Gurley *et al*., 2005, 2008). P-gp ATPase activity increased when incubated with goldenseal extract, berberine, or hydrastine, indicating an effect on the P-gp transport system (Etheridge *et al*., 2007).

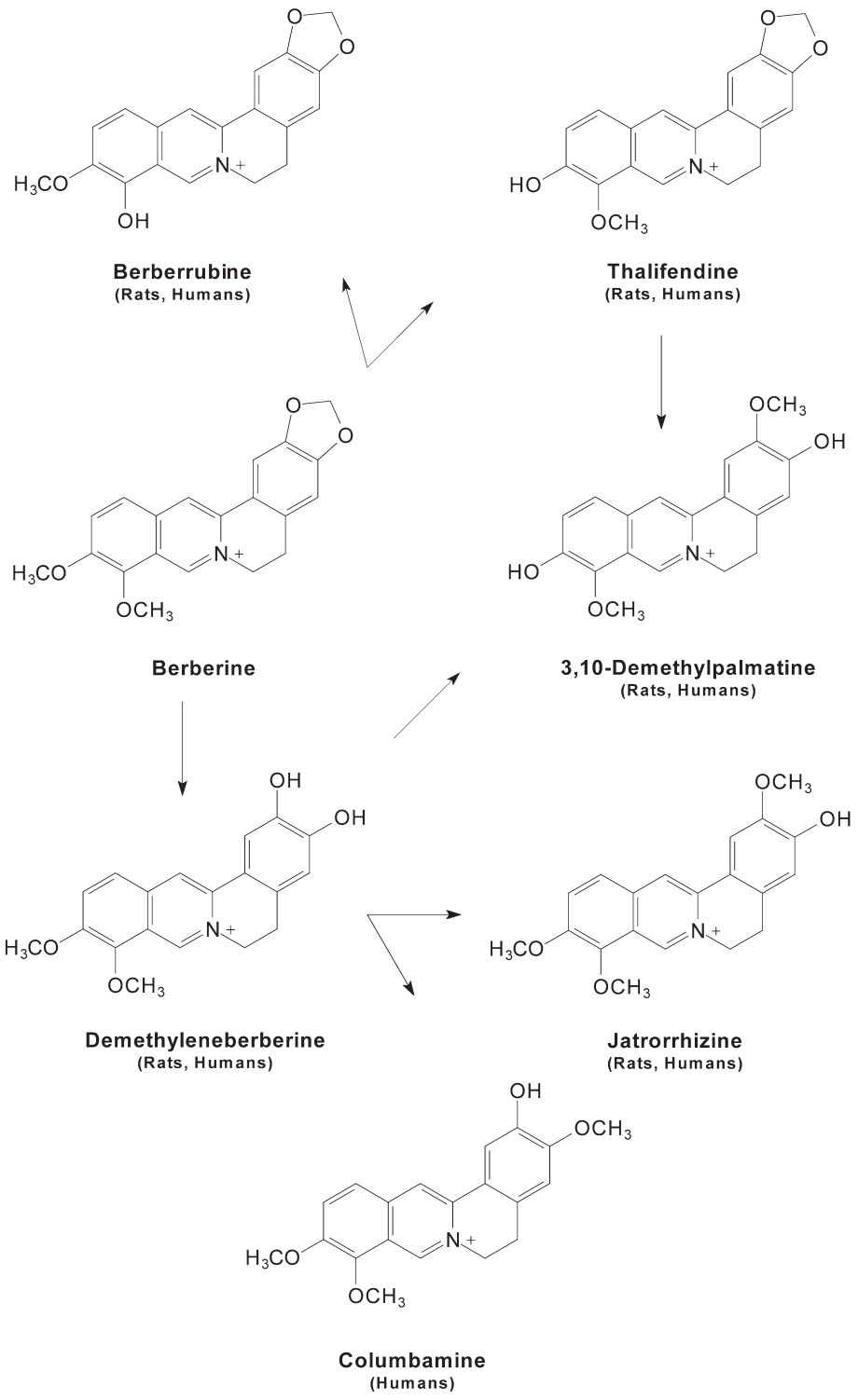


FIGURE 2
Proposed Metabolic Scheme for Berberine in Rats and Humans
(Adapted from Pan *et al*., 2002; Zuo *et al*., 2006; and Qiu *et al*., 2008)

TOXICITY

Goldenseal

No 14-day or 90-day toxicity studies of goldenseal or its alkaloids have been reported in the literature. Oral LD_{50} values have not been reported for goldenseal.

Goldenseal Alkaloids

LD_{50} values for berberine and hydrastine are shown in Table 1.

Experimental Studies

Goldenseal Alkaloids

Most of the rodent studies of berberine reported in the literature were designed to look for beneficial effects of berberine. Pretreatment of rats with berberine (4 mg/kg, orally twice daily for 2 days) was reported to prevent acetaminophen- or carbon tetrachloride-induced increases in serum levels of alkaline phosphatase and aminotransaminases (Janbaz and Gilani, 2000). Berberine administered to male Wistar rats (125 mg/kg twice a day for 5 weeks) fed a high-fat diet caused an increased insulin sensitivity as measured by reduced blood glucose levels (Yin *et al*., 2008). In two studies, berberine sulfate at 0.1 to 6.0 mg/kg (0.00023-0.014 mmol/kg) (Sabir and Bhide, 1971) or 0.15 to 0.30 mg/kg (0.00035-0.00069 mmol/kg) (Sabir *et al.*, 1978) reduced the blood pressure of rats (strain not provided). One study showed that berberine (100 mg/kg) altered exploratory behavior in mice by increasing the time spent in a maze test (Peng *et al*., 2004).

In vitro studies have been targeted at identifying biological activities of berberine, including anticancer and cardioprotective activities and effects on specific enzyme activities (Lau *et al*., 2001; Mazzini *et al*., 2003; Leng *et al*., 2004; Peng *et al*., 2004; Jantová *et al*., 2007; Serafim *et al*., 2008), but few studies have been conducted to confirm these results *in vivo* or in the clinic (Mar and Bent, 1999). Berberine was shown to possess cytotoxic activity via induction of apoptosis in human uterus HeLa cells and murine leukemia L1210 cell lines (Kettmann *et al*., 2004a); immunoregulative activity by induction of interleukin-12 in mouse macrophages (Kim *et al*., 2003); anti-inflammatory activity against serotonin-induced edema in mice (Küpeli *et al*., 2002); and antioxidative activity in heterogeneous liposome membrane systems stressed by peroxidative damage (Račková *et al*., 2004). Several studies suggest that berberine may have cardioprotective activity causing vascular relaxation [antihypertensive effects in isolated rat aortas (Lee and Chang, 1996) or in rat mesenteric arteries (Ko *et al*., 2000)], vasorelaxing effects in rat isolated mesenteric arteries exposed to phenylephrine (Ko *et al*., 2000), and cardioprotective activity to hypoxia in myocytes from neonatal Sprague-Dawley rats (Zheng *et al*., 2003). Berberine has been reported to alter enzyme activity (NADH oxidase, reverse transcriptase,

TABLE 1
Summary of Selected Acute Animal Toxicity Data for Berberine and Hydrastine

	Species	Route of Administration	LD_{50} (mg/kg)	Reference
Berberine				
	Mouse	Oral	329	RTECS, 1997
	Mouse	Subcutaneous	18	RTECS, 1997
Berberine Sulfate				
	Rat	Intramuscular	14.5	Kowalewski *et al.*, 1975
	Rat (albino)	Intraperitoneal	205	Kulkarni *et al.*, 1972
	Rat	Intraperitoneal	88.5	Kowalewski *et al.*, 1975
	Mouse (male and female albino)	Intraperitoneal	24.3	Sabir and Bhide, 1971
	Rat	Oral	>1,000	Kowalewski *et al.*, 1975
Hydrastine				
	Rat	Intraperitoneal	104	RTECS, 1997

diaminooxidase, topoisomerase, activated protein 1, and cyclooxygenase-2) in human promonocytic U937 cells (Jantová *et al*., 2006). Berberine induced apoptosis through a mitochondria/caspase pathway in human hepatoma cells (Hwang *et al.*, 2006). Berberine reduced cytotoxicity in human umbilical vein endothelial cells exposed to oxidized lipids (Hsieh *et al*., 2007).

Humans

Goldenseal

There is limited information in the literature on the potential toxicity of goldenseal in humans. Anecdotal reports of oral exposures to goldenseal have been reported to cause convulsions, irritation of the mouth, throat, and stomach in humans (Hamon, 1990).

Goldenseal is reported to inhibit serum activity of cytochrome P450s (2D6 and 3A4/5) after oral administration (900 mg three times daily; estimated daily dose of 77 mg berberine and 68 mg of hydrastine) to volunteers for 28 days (Gurley *et al.*, 2005). In a follow up study in healthy volunteers (Gurley *et al*., 2008), goldenseal (1,070 mg, three times daily for 14 days; equal to 77 mg berberine per day) was reported to disrupt the metabolism of debrisoquine (5 mg), a chemical known to be metabolized by CYP2D6 (Streetman *et al*., 2000), based on the content of debrisoquine and 4-hydroxydebrisoquine in urine.

Reproductive and Developmental Toxicity

Experimental Animals

Goldenseal

Developmental toxicity of goldenseal was examined after administration of goldenseal in feed to Sprague-Dawley rats at doses of 0, 3,121, 6,250, 12,500, or 18,400 ppm from gestation day 6 to 20 (NTP, 2003b) and Swiss CD-1 mice at doses of 0, 3,121, 12,500, or 50,000 ppm from gestation day 6 to 17 (NTP, 2002a). Maternal evaluations included: body, liver, and gravid uterus weights; examination of thoracic and abdominal cavities, and maternal organs for gross lesions; ovarian corpora lutea count; uterine examination to determine the number of implantation sites, resorptions, dead, and live fetuses; and determination of implantation sites using ammonium sulfide (10%) stain. Embryo/fetal evaluations included count, body weight, and sex determination for live pups; examination for internal, skeletal, and external malformations. Maternal or pup toxicity was restricted to increased dam liver weights and/or decreases in pup body weights.

In rats (NTP, 2003b), maternal liver weights were increased at goldenseal exposures of 6,250 ppm and greater. At the highest exposure (18,400 ppm), average absolute liver weight reached 113% of the average control weight. There were no treatment-related effects on prenatal mortality (resorptions and/or late fetal deaths), average live litter size, average fetal body weight per litter (male, female, or both sexes), or percent male fetuses per litter. There were no treatment-related malformations in the fetuses (i.e., external, visceral, or skeletal). There was no definitive evidence of developmental toxicity in this goldenseal rat study.

In the mouse goldenseal feed study (NTP, 2002a), as in the rat study, the primary effect noted was an increase in maternal liver weight at 12,500 and 50,000 ppm. At 50,000 ppm, mean absolute maternal liver weight reached 140% of the control value. However, no liver lesions were seen in a select group of treated dams (10 heaviest maternal livers per group revealed no treatment-related lesions). Prenatal mortality, average live litter size, and percent male fetuses per litter were not affected. Female fetal body weight/litter exhibited a decreasing trend with a 6.5% reduction (not statistically significant) at 50,000 ppm. Fetal body weight/litter (males or both sexes combined) exhibited a significant reduction (8%) at 50,000 ppm. There were no statistically significant increases in the incidences of fetal malformations. The incidences of exencephaly and cleft palate appeared to be slightly increased at 50,000 ppm and exceeded the historical control range (1.0% vs. 0.7%), but this difference was not statistically significant when compared to the concurrent control group.

No maternal or fetal toxicity was seen in female Sprague-Dawley rats administered an ethanol extract of goldenseal at 1.86 g/kg per day on gestation days 1 to 8 or gestation days 8 to 15 (Yao *et al*., 2005).

Goldenseal Alkaloid: Berberine

Developmental toxicity of berberine, given in the feed or by oral gavage, was examined in Sprague-Dawley rats and Swiss CD-1 mice (NTP, 2002b; NTP, 2003a,c,d; Jahnke *et al*., 2006). In the feed studies (NTP 2003c,d),

rats were exposed to 0, 3,625, 7,250, or 14,500 ppm berberine hydrochloride dihydrate (0, 282, 531, or 1,313 mg/kg per day, respectively) on gestation days 6 to 20 and mice were exposed to 0, 3,500, 5,250, or 7,000 ppm (0, 569, 841, or 1,155 mg/kg per day, respectively) on gestation days 6 to 17. There were some treatment-related decreases in maternal weight gain and/or fetal body weights compared to the respective controls, but in contrast to the goldenseal developmental toxicity studies, there were no increases in maternal liver weights. There was no evidence for treatment-related effects on number of implantation sites or average litter size, and there was no evidence for any treatment-related pup malformation in either the rat or mouse study (Jahnke *et al*., 2006).

Berberine hydrochloride dihydrate was also administered to Swiss CD-1 mice and Sprague-Dawley rats by oral gavage (the vehicle was 0.5% methylcellulose) at doses of 0 or 1,000 mg/kg per day on gestation days 6 to 19 (rats) or 6 to 16 (mice) (NTP, 2002b, 2003a). In the rat study, there were no statistically significant treatment-related decreases in dam body weight gain or in pup body weight. In the mouse study, there was also no effect on dam body weight gain, although male average fetal body weight was lower than in the controls. The primary effect noted in the berberine hydrochloride dihydrate mouse study was early death of dams (33% of dams given 1,000 mg/kg per day died or were sacrificed moribund). Early death of dams was not seen in the rat study. There were no treatment-related effects in the other reproductive parameters evaluated in either the rat or mouse gavage study, including no effect on number of ovarian corpora lutea per dam, number of implantation sites, or average litter size. There were no treatment-related malformations in either the mouse or rat pups (Jahnke *et al*., 2006).

Humans

No studies analyzing reproductive or developmental toxicity of goldenseal in humans are reported in the literature.

CARCINOGENICITY

Experimental Animals

There are no 2-year rodent carcinogenicity studies of goldenseal, berberine, or hydrastine reported in the literature.

Anticarcinogenicity studies reported in the literature have examined potential effects of berberine. Berberine is reported to have cytotoxic/antiproliferative effects in various cancer cell lines (Chi *et al*., 1994; Jantová *et al*., 2006). Berberrubin (9-demethylberberine) prevented growth of sarcoma-180 ascites cells, while berberine and tetrahydroberberine showed no such inhibition of cell growth (Hoshi *et al*., 1976). Berberine (12.5 to 50 µM) promoted G1 arrest in K1735-M2 mouse and WM793 human melanoma cells and G2 arrest at higher dose levels (Serafim *et al*., 2008). Berberine suppressed lipopolysaccharide, 12-*O*-tetradecanoylphorbol-13-acetate, or hydrogen peroxide-induced inflammation in human lung epithelial cells (A-549) (Lee *et al*., 2007). Berberine has been shown to induce apoptosis in human promonocytic cells (Jantová *et al*., 2007).

Oral administration of berberine hydrochloride (0.5, 2.5, or 5 mg/kg) three times a week for 5 weeks prior to a single dermal application of 20-methylcholanthrene, and then three times a week for 8 weeks reduced the incidence of sarcomas in Swiss albino mice observed for up to 180 days (Anis *et al*., 2001). Berberine administered orally to female Wistar rats (25 or 50 mg/kg, 5 days per week for 20 weeks) 24 hours before oral administration of *N*-nitrosodiethylamine (NDEA); 5 days per week for 20 weeks, followed by 10 weeks with no dosing, reduced the incidence of NDEA-induced hepatocarcinogenesis (pathology details not given; Anis *et al*., 2001). Administration of berberine (200 mg/kg per day) to Balb/c nude mice for 7 days prior to injection of nasopharyngeal carcinoma cells reduced the amount of implanted tumor measured 4 weeks later (Liu *et al*., 2008).

In a two-stage mouse skin carcinogenicity study, berberine sulfate significantly inhibited the tumor yield and the incidence of tumor-bearing animals initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA) and promoted with teleocidin (Nishino *et al*., 1986). In this study, female ICR mice were initiated with a single application of DMBA onto dorsal skin. Starting 1 week after the DMBA dose, teleocidin was applied dermally twice per week for 18 weeks; berberine sulfate (0.5 mg; 0.0012 mmol) dissolved in ethanol/dimethyl sulfoxide was also applied dermally 40 minutes before each teleocidin application. At week 18, approximately 85% of the vehicle controls had tumors, whereas only approximately 12% of berberine-sulfate-treated mice did. Berberine sulfate alone was not administered to any animal.

Zhang *et al*. (1990) evaluated the anticarcinogenic activity of berberine and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in adult male Fischer 344 rats with 9L or BCNU-resistant 9L-2 rat gliosarcoma cells implanted in the brain. Rats bearing 9L tumor cells were treated intraperitoneally, 14 to 15 days after implantation, with berberine alone (10 mg/kg; 0.030 mmol/kg), BCNU alone (4.43 mg/kg), or a combination of berberine and BCNU at these doses. Rats bearing 9L-2 tumor cells were similarly treated but received a higher dose of BCNU (6.66 mg/kg). Rats were killed 24 hours after treatment. Brain tumors were removed and converted into single-cell suspensions that were incubated for 12 to 14 days. In 9-L tumor-bearing rats, berberine alone and BCNU alone achieved 81% and 76% cell death, respectively. Treatment with both compounds produced 95.2% cell death. In 9L-2 tumor-bearing rats, berberine alone was inactive.

Humans

Goldenseal

No controlled epidemiological studies examining potential cancer effects of goldenseal (or its alkaloid components) are reported in the literature. There have been incidental side effects from goldenseal, although in general, these side effects have not been documented in controlled trials with sufficient numbers (NSD, 2007a).

Goldenseal Alkaloid: Berberine

Berberine, a component of goldenseal, has been considered as a possible chemotherapeutic drug for cancer or psoriasis (Maiti and Kumar, 2007; Grycová *et al*., 2007).

Genetic Toxicity

Goldenseal

No reports on the genotoxicity of goldenseal were found in the literature.

Goldenseal Alkaloids

Several studies examined the mutagenicity and DNA reactivity of berberine compounds. Weakly mutagenic activity was reported for berberine hydrochloride in *Salmonella typhimurium* strain TA98 in the absence of exogenous metabolic activation, but no mutagenicity was observed in strain TA100, with or without metabolic activation (Nozaka *et al*., 1990). No mutagenic activity was observed with berberine chloride in the SOS chromotest in *Escherichia coli* strain PQ37, with or without S9, and no genotoxicity was observed in *Saccharomyces cerevisiae* strains treated during non-growth stages (Pasqual *et al*., 1993). However, increases in frameshift mutations and mitotic recombination were observed in both DNA-repair proficient and deficient strains of *S. cerevisiae* when treatment with berberine chloride occurred during mitotic growth stages (Pasqual *et al*., 1993). The authors suggested that the observed genotoxic effects of berberine chloride in the yeast test system resulted from its intercalation into yeast DNA and subsequent disruption of topoisomerase enzyme activities.

Evidence for the complexing of berberine and other related alkaloids to DNA via intercalation, preferentially at AT-rich sequences, was recently reviewed by Maiti and Kumar (2007). Additional studies have supported the ability of berberine to bind to DNA via partial intercalation, with a strong affinity for AT-rich regions (Bhadra *et al*., 2008). Mazzini *et al*. (2003) provided evidence that berberine, rather than intercalating, binds to AT-rich DNA regions within the minor groove. Minor changes in structure of protoberberine alkaloids can have a dramatic effect on binding. Pilch *et al*. (1997) proposed a mixed binding model that incorporated both intercalation and minor groove binding and depended upon substitution at key binding positions.

The ability of berberine to induce DNA damage was confirmed in a number of studies. Berberine chloride (150 µg/mL) was reported to significantly increase the frequency of sister chromatid exchanges, indicators of DNA damage, in cultured rat gliosarcoma cells (Zhang *et al*., 1990). Jantová *et al*. (2006) reported that berberine chloride induced DNA single strand breaks, detected by the Comet Assay, in NIH-3T3 cells at concentrations of 0.27 to 2.69 µM and in Ehrlich ascites carcinoma cells over a range of 0.14 to 0.67 µM; similar levels of dose-related increases in DNA damage were observed with

and without concomitant UV radiation. In contrast to the findings reported by Jantová *et al*. (2006), Inbaraj *et al*. (2001) observed no induction of DNA damage, measured by the Comet Assay, in HaCaT keratinocytes (transformed human epidermal cells) exposed to berberine chloride over a concentration range of 10 to 50 µM in the dark; however, the authors reported that cotreatment of the cells with UV radiation induced a concentration-related increase in DNA damage at berberine chloride concentrations of 25 and 50 µM. Co-treatment of HaCaT keratinocytes with ultraviolet radiation and hydrastine or canadine (minor constituent alkaloids of goldenseal) did not result in increased levels of DNA damage detected by the Comet assay (Inbaraj *et al*., 2006).

Study Rationale

Goldenseal was nominated by the National Institute of Environmental Health Sciences for toxicity and cancer studies based on the potential for human exposure and the lack of carcinogenicity data, and because it is one of the most widely used herbs in the United States.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF GOLDENSEAL ROOT POWDER

Goldenseal root powder was obtained from Plantation Medicinals, Inc. (Felda, FL), in one lot (007-090200) used for the 2-week and 3-month studies. Goldenseal roots were purchased from Strategic Sourcing, Inc. (Reading, PA), in one lot (HYCA 10/7-10.28.01-C) that was used for the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO) and by the study laboratories at BioReliance Corporation (Rockville, MD; 2-week studies) and Southern Research Institute (Birmingham, AL; 3-month and 2-year studies) (Appendix I). Nutritional, contaminant, and microbiological tests were conducted by Covance Laboratories, Inc. (Madison, WI). Stability analyses of the bulk chemical were conducted by the analytical chemistry laboratory. Reports on analyses performed in support of the goldenseal root powder studies are on file at the National Institute of Environmental Health Sciences.

Goldenseal root powder (lot 007-090200) was identified by its color and alkaloid profile as described in the literature (AHP, 2001). Goldenseal root (HYCA 10/7-10.28.01-C) was also identified by its distinctive appearance prior to grinding. Characteristically, goldenseal root powder contains no less than 2.0% hydrastine and 2.5% berberine on a dry weight basis. Both lots were milled so that at least 75% of the resulting material passed through a 60-mesh (250 µm) sieve. Identity was further confirmed from the profile and presence of the alkaloids berberine, hydrastine, and canadine together, which is characteristic of goldenseal root, and the absence of palmatine, which is characteristic of coptis, a common adulterant of goldenseal products.

The purities of acetonitrile:water:phosphoric acid (70:30:0.1) extracts of lots 007-090200 and HYCA 10/7-10.28.01-C were determined using high-performance liquid chromatography (HPLC). The purity of lot HYCA 10/7-10.28.01-C was also determined by thin-layer chromatography (TLC), headspace and extract analyses by gas chromatography coupled with mass spectrometry (GC/MS), liquid chromatography coupled with mass spectrometry (LC/MS), and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF/MS).

HPLC indicated that lot 007-090200 contained 3.45% berberine, 3.02% hydrastine, and 0.08% canadine by weight; palmatine was not detected. An aliquot of lot 007-090200 was submitted to Covance Laboratories, Inc. for nutritional and contaminant testing using standard methods. Organochlorine and organophosphorus pesticide levels were below the detection limit except for captan, which was present at 113 ppb. Aflatoxin and nitrosamine levels were below the detection limit of 1 ppb and mercury was below the detection limit of 25 ppb. Lead and selenium were present at 204 and 3.8 ppb, respectively. Microbial tests were not performed on lot 007-090200.

For lot HYCA 10/7-10.28.01-C, weight loss on drying indicated a moisture content of 6.35%. Hydrastinine, hydrastine, and berberine were visualized by TLC, but palmatine was not observed. Additional HPLC analyses using the same system indicated that palmatine, berberine, hydrastine, canadine, and total alkaloids were present at 0.0%, 3.89%, 2.80%, 0.17%, and 6.86%, respectively, and the B/H ratio was determined to be 2.84. By comparison with a mass spectral library, 17 volatile organic components were tentatively identified by GC/MS in the goldenseal root powder headspace samples and five components (including hydrastinine, hydrastine, and canadine) were tentatively identified in the test article extracts. LC/MS analyses of goldenseal root powder extracts did not detect palmatine but did identify hydrastinine, hydrastine, berberine, and canadine, as well as the known goldenseal alkaloids tetrahydroberberastine, canadaline, and berberastine. MALDI-TOF/MS analyses detected ions corresponding to hydrastine, berberine, and several unidentified components in all of the goldenseal extracts and ions corresponding to hydrastidine and/or canadaline only in the acetonitrile:water:phosphoric acid (70:30:0.1) extract. An aliquot of lot HYCA 10/7-10.278.91-C was submit-

ted to Covance Laboratories, Inc. for nutritional and contaminant testing using standard methods. Organochlorine and organophosphorus pesticide levels were below the detection limit, except for pentachloronitrobenzene (PCNB), which was present at 218 ppb. Lead, arsenic, and selenium were present at 450, 130, and 45 ppb, respectively. Mercury levels were below the detection limit of 25 ppb. Aflatoxin and nitrosamine levels were below the detection limit of 1 ppb. Microbial testing results were as follow: standard plate count, 11,000 CFU/g; total coliform, 43 MPN/g; *Salmonella typhimurium*, negative per 25g; mold count, 35 col/g; fecal coliform less than 3.0 MPN/g, *Escherichia coli* less than 10 CFU/g; and yeast count, less than 10 col/g. Results of the nutritional, contaminant, and microbiological tests were deemed acceptable for use in these studies.

Stability studies of the bulk chemical were performed by monitoring the palmatine, berberine, hydrastine, canadine, and total alkaloids content of the test article. Samples of the test article were extracted with acetonitrile:water:phosphoric acid (70:30:0.1) and the extracts were analyzed by HPLC. These studies indicated that goldenseal root powder was stable as a bulk chemical for at least 2 weeks when stored protected from light at temperatures up to 25° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in plastic bags. Periodic reanalyses of the bulk chemical were performed using HPLC; no degradation of the bulk chemical was detected by measuring the B/H area ratios.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing goldenseal root powder with feed (Table I1).

Homogeneity studies of 1,560 and 50,000 ppm dose formulations and of 3,121 and 50,000 ppm dose formulations were performed by BioReliance Corporation and Southern Research Institute, respectively. These studies were conducted with HPLC and measured the berberine content of acetonitrile:water:phosphoric acid (70:30:0.1) extracts of the dose formulations. These analytical procedures were also used in stability studies of a 1,500 ppm dose formulation of lot 8147 that were performed by the analytical chemistry laboratory. Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored in opaque double-thickness plastic bags under freezer, refrigerated, and room temperature conditions; stability was also confirmed for at least 17 days under simulated animal room conditions.

Periodic analyses of the dose formulations of goldenseal root powder were conducted by the study laboratories using HPLC. All determinations of the concentrations of goldenseal root powder in feed were based on quantification of peak areas produced by the marker compound berberine. During the 2-week studies, the dose formulations were analyzed once; all six of the dose formulations for rats and mice were within 10% of the target concentrations (Table I2). Animal room samples of these dose formulations were also analyzed; four of six for rats and one of six for mice were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed at the beginning and at the end of the studies; animal room samples were also analyzed (Table I3). All 40 of the dose formulations analyzed for rats and mice were within 10% of the target concentrations; all 10 of the animal room samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 8 to 12 weeks (Table I4). Of the dose formulations analyzed, all 173 for rats and all 85 for mice were within 10% of the target concentrations; all 15 of the animal room samples for rats and 11 of 12 for mice 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 14 (rats) or 15 (mice) days and were 6 to 7 weeks old on the first day of the studies. Groups of five male and five female rats and mice were fed diets containing 0, 1,560, 3,121, 6,250, 12,500, 25,000, or 50,000 ppm goldenseal root powder for 15 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings were recorded daily and feed consumption was recorded on days 8 and 15 of the studies. The animals were weighed initially, on study day 8, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Necropsies were performed on all rats and mice. The brain, heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations of the liver were performed on control and 50,000 ppm rats and mice; gross lesions were examined microscopically in the remaining groups to a no-effect level. 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 goldenseal root powder and to determine the appropriate exposure concentrations 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 to 12 days (rats) or 13 to 14 days (mice) 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. At the end of the studies, serologic analyses were performed on five male and five female control rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix L).

Groups of 10 male and 10 female rats and mice were fed diets containing 0, 3,121, 6,250, 12,500, 25,000, or 50,000 ppm goldenseal root powder for 14 weeks. Additional groups of 10 male and 10 female clinical pathology rats were given the same concentrations for 23 days. Feed and water were available *ad libitum*. Rats and female mice were housed 5 per cage; male mice were housed individually. Clinical findings were recorded weekly for core study rats and mice. Feed consumption was recorded weekly by cage except during week 1 when consumption was measured over 1 to 4 days (female mice), 3 days (male rats), 2 days (male mice), or 4 days (female rats). Core study animals were weighed on day 1, day 8 or 9, then weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Blood was collected from the retroorbital sinus of clinical pathology rats on days 5 and 23 and from core study animals at the end of the studies for hematology and clinical chemistry analyses (rats only). Animals were anesthetized with a CO_2/O_2 mixture. Blood for hematology was collected into a tube containing EDTA, and blood for clinical chemistry was collected into tubes containing no anticoagulant. Reagents were manufactured or supplied by Bayer, Inc. (Tarrytown, NY), Sigma Diagnostics (St. Louis, MO), Boehringer-Mannheim Corporation (Indianapolis, IN), Roche Diagnostics (Indianapolis, IN), or Fisher Scientific (Norcross, GA). Blood smears were prepared within approximately 2 hours of sample collection. Blood smears for hematology were stained using a modified Wright's stain and the Ames HEMA-TEK® slide stainer, and parameters were measured using an ADVIA 120 Hematology System Analyzer (Bayer, Inc.). Platelet and erythrocyte morphologies were examined by light microscopy. Clinical chemistry parameters were evaluated using a Hitachi 911 Chemistry Analyzer (Roche Diagnostics Corporation, Indianapolis, IN). The parameters measured are listed in Table 2.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats and mice exposed to 0, 12,500, 25,000, or 50,000 ppm. 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.

TABLE 2
Experimental Design and Materials and Methods in the Feed Studies of Goldenseal Root Powder

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory		
BioReliance Corp. (Rockville, MD)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
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		
Rats: 14 days Mice: 15 days	Rats: 11 (females) or 12 (males) days Mice: 13 (males) or 14 (females) days	12 days
Age When Studies Began		
6 to 7 weeks	5 to 6 weeks	5 to 6 weeks
Date of First Exposure		
Rats; June 21, 2001 Mice: June 22, 2001	Rats: January 27 (females) or 28 (males), 2002 Mice: January 29 (males) or 30 (females), 2002	Rats: April 21, 2003 Mice: May 12, 2003
Duration of Exposure		
15 days	14 weeks	105-106 weeks
Date of Last Exposure		
Rats: July 05, 2001 Mice: July 06, 2001	Rats: Core study: April 30 (females) or May 01 (males), 2002 Clinical pathology study: February 18 (females) or 19 (males), 2002 Mice: May 02 (males) or 03 (females), 2002	Rats: April 18 through 25, 2005 Mice: May 9 through 16, 2005
Necropsy Dates		
Rats: July 05, 2001 Mice: July 06, 2001	Rats: April 30 (females) or May 01 (males), 2002 Mice: May 02 (males) or 03 (females), 2002	Rats: April 18 through 25, 2005 Mice: May 9 through 16, 2005
Age at Necropsy		
8 to 9 weeks	19 to 20 weeks	Rats: 110 weeks Mice: 109 to 111 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

TABLE 2
Experimental Design and Materials and Methods in the Feed Studies of Goldenseal Root Powder

2-Week Studies	3-Month Studies	2-Year Studies
Animals per Cage		
Rats: 5 Mice: 1 (males), 5 (females)	Rats: 5 Mice: 1 (males), 5 (females)	Rats: 3 (males), 5 (females) Mice: 1 (males), 5 (females)
Method of Animal Identification		
Tail tattoo	Tail tattoo	Tail tattoo
Diet		
Irradiated NTP-2000 open formula meal (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 2-week studies	Same as 2-week studies
Water		
Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Tap water (Birmingham, AL, municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 3-month studies
Cages		
Polycarbonate (Lab Products, Inc., Seaford, DE), changed once (male mice) or twice (rats and female mice) weekly	Polycarbonate (Lab Products, Inc., Maywood, NJ), changed once (male mice) or twice (rats and female mice) weekly	Same as 3-month studies
Bedding		
Irradiated heat-treated, Sani-Chip hardwood bedding (P.J. Murphy Forest Products, Montville, NJ), changed once (male mice) or twice (rats and female mice) weekly	Same as 2-week studies	Same as 2-week studies
Cage Filters		
Remay 2016 (Snow Filtration, West Chester, OH), changed once	Reemay spun-bonded polyester (Andico, Birmingham, AL), changed every two weeks	Same as 3-month studies
Racks		
Stainless steel (Lab Products, Inc., Seaford, DE), changed once	Same as 2-week studies, changed every 2 weeks	Same as 3-month studies
Animal Room Environment		
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	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
Exposure Concentrations		
0, 1,560, 3,121, 6,250, 12,500, 25,000, or 50,000 ppm in feed, available *ad libitum*	0, 3,121, 6,250, 12,500, 25,000, or 50,000 ppm in feed, available *ad libitum*	0, 3,000, 9,000, or 25,000 ppm in feed, available *ad libitum*

TABLE 2
Experimental Design and Materials and Methods in the Feed Studies of Goldenseal Root Powder

2-Week Studies	3-Month Studies	2-Year Studies
Type and Frequency of Observation		
Observed twice daily; animals were weighed on day 1, on day 8, and at the end of the studies; clinical findings were recorded daily. Feed consumption was recorded on days 8 and 15.	Observed twice daily; core study animals were weighed on day 1, day 8 or 9, then weekly, and at the end of the studies; clinical findings were recorded weekly. Feed consumption for 7-day periods was recorded weekly by cage except during week 1 when consumption was measured over 1 to 4 days (female mice), 3 days (male rats), 2 days (male mice), or 4 days (female rats).	Observed twice daily; clinical findings were recorded monthly; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies. Feed consumption was recorded weekly for 13 weeks, then monthly.
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 brain, heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of clinical pathology rats on days 5 and 23 and from core study animals at the end of the studies for hematology and clinical chemistry (rats only). ***Hematology:*** automated hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated 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, and bile acids	None

TABLE 2
Experimental Design and Materials and Methods in the Feed Studies of Goldenseal Root Powder

2-Week Studies	3-Month Studies	2-Year Studies
Histopathology		
Liver histopathology was performed on control and 50,000 ppm rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice only), harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, 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 control and 50,000 ppm rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice only), harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, 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 all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice only), harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, 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 animals in the 0, 12,500, 25,000, and 50,000 ppm 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 females exposed to 0, 12,500, 25,000, and 50,000 ppm for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's Solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on control and 50,000 ppm rats and mice; tissues were examined to a no-effect level in the remaining exposure groups. Table 2 lists the tissues and organs routinely examined.

2-Year Studies

Study Design

Groups of 50 male and 50 female rats and mice were fed diets containing 0, 3,000, 9,000, or 25,000 ppm goldenseal root powder for 105 to 106 weeks.

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. Rats and mice were quarantined for 12 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 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L).

Animal Maintenance

Rats were housed 3 (males) or 5 (females) per cage and mice were housed individually (males) or 5 (females) per cage. Feed and water were available *ad libitum*. Feed consumption was measured weekly for 13 weeks and then monthly until the end of the studies. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded monthly and body weights were recorded initially, weekly for 13 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 (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the liver of rats and mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (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 or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C4, 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., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables 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 exposed group with controls and a test for an overall exposure-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 exposure concentrations. 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 toxicities of goldenseal root powder and berberine chloride were assessed by testing the ability of these chemicals to induce mutations in various strains of *Salmonella typhimurium, Escherichia coli,* micronucleated erythrocytes in mouse peripheral blood, and micronucleated erythrocytes in mouse bone marrow. 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 (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. 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). Final mean body weights and body weight gains of exposed animals were similar to those of the controls. Feed consumption by exposed groups was similar to that by the controls. Dietary concentrations of 1,560, 3,121, 6,250, 12,500, 25,000, and 50,000 ppm resulted in average daily doses of approximately 155, 315, 630, 1,190, 2,465, and 4,815 mg goldenseal root powder/kg body weight for males and average daily doses of 150, 290, 640, 1,240, 2,370, and 4,870 mg/kg for females. No clinical findings attributed to goldenseal root powder exposure were observed.

Absolute and relative liver weights of males exposed to 6,250 ppm or greater and females exposed to 12,500 ppm or greater were significantly greater than those of the controls and relative liver weights were significantly increased in males exposed to 1,560 or 3,121 ppm and females exposed to 6,250 ppm (Table G1). The liver weights of exposed groups of males were 109%, 111%, 123%, 132%, 141%, and 149% that of the controls for the 0, 1,560, 3,121, 6,250, 12,500, 25,000, and 50,000 ppm groups, respectively. The liver weights of exposed groups of females were 98%, 105%, 110%, 117%, 122%, and 137% that of the controls. Minimal to moderate hepatocellular hypertrophy was noted in three males and all females exposed to 25,000 ppm and in all 50,000 ppm males and females [males: 0 ppm, 0/5;

TABLE 3
Survival, Body Weights, and Feed Consumption of Rats in the 2-Week Feed Study of Goldenseal Root Powder

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	139 ± 3	209 ± 5	70 ± 3		18	19
1,560	5/5	141 ± 3	212 ± 3	72 ± 2	101	18	20
3,121	5/5	140 ± 2	212 ± 4	72 ± 4	101	18	21
6,250	5/5	132 ± 3	210 ± 5	78 ± 3	100	18	20
12,500	5/5	140 ± 2	210 ± 3	69 ± 2	100	17	19
25,000	5/5	138 ± 4	204 ± 6	66 ± 3	98	18	19
50,000	5/5	135 ± 6	201 ± 8	65 ± 2	96	17	19
Female							
0	5/5	113 ± 3	145 ± 3	33 ± 2		14	14
1,560	5/5	112 ± 3	141 ± 2	29 ± 3	97	13	13
3,121	5/5	118 ± 3	145 ± 3	27 ± 1	100	13	13
6,250	5/5	111 ± 4	139 ± 4	28 ± 1	96	13	14
12,500	5/5	111 ± 3	142 ± 3	31 ± 3	98	13	14
25,000	5/5	110 ± 2	143 ± 2	32 ± 1	98	12	13
50,000	5/5	112 ± 3	142 ± 4	30 ± 2	98	13	13

[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the control group are not significant by Dunnett's test.
[c] Feed consumption is expressed as grams per animal per day.

1,560 ppm, 0/0; 3,121 ppm, 0/1; 6,250 ppm, 0/0; 12,500 ppm, 0/5; 25,000 ppm, 3/5 (1.0); 50,000 ppm, 5/5 (2.3); females: 0/5; 0/1; 0/0; 0/0; 0/5; 5/5 (1.0); 5/5 (2.2)]. Hepatocellular hypertrophy was characterized by enlargement of the hepatocytes in the centrilobular areas of the lobules.

Exposure Concentration Selection Rationale: In the 2-week study all animals survived and final mean body weights of the 50,000 ppm groups were within 4% of those of the controls. The severity of hepatocyte centrilobular hypertrophy and the increase in liver weights of the exposed groups were not considered severe enough to compromise the 3-month study. The exposure concentrations selected for the 3-month study in rats were 3,121, 6,250, 12,500, and 50,000 ppm.

3-Month Study

All rats survived to the end of the study (Table 4). Final mean body weights in this study were the last in-life weights on day 85 (males) or day 86 (females); none of the final body weights or body weight changes in any exposed group was significantly different from those of the controls. Feed consumption by exposed groups was generally similar to that by controls. Dietary concentrations of 3,121, 6,250, 12,500, 25,000, and 50,000 ppm resulted in average daily doses of approximately 255, 500, 1,000, 2,020, and 4,060 mg/kg body weight for males and 260, 500, 1,030, 2,070, and 4,100 mg/kg for females. No clinical findings related to goldenseal root powder exposure were observed.

The hematology and clinical chemistry data for rats in the 3-month toxicity study of goldenseal root powder are shown in Table F1. On day 5, an increase in the erythron, evidenced by increases in the hematocrit, hemoglobin, and erythrocyte counts, occurred in 25,000 ppm females and 50,000 ppm males and females. The erythron increases were transient, occurring only on day 5, and minimal ($\leq$7%) and were consistent with a transient physiologic hemoconcentration possibly related to a transient decrease in water intake (dehydration) early in the study. The transient decrease in reticulocyte counts on day 5 were consistent with the increased erythron.

At week 14, serum chemistry evaluations demonstrated exposure-related decreases in activities of alkaline phosphatase, alanine aminotransferase, and sorbitol dehydrogenase in most exposed groups of males and/or females; alkaline phosphatase was affected on day 23. The significance or mechanism for the decreases in serum activity of these enzymes are unknown. However, it has been reported that rats treated with 4 mg/kg berberine twice daily for two days prevented acetaminophin- or carbon tetrachloride-induced increases in alkaline phosphatase and alanine aminotransferase activity (Janbaz and Gilani, 2000). In males and females, small, but progressive, increases in total protein concentrations occurred in 12,500 ppm or greater groups and in serum albumin in the 25,000 ppm or greater groups; the mechanism was unknown. A true overproduction of albumin is not known to occur in animals (Kaneko, 1989), however, any increase in serum concentration of albumin is interpreted as dehydration.

Absolute and relative liver weights were significantly increased in males exposed to 6,250 ppm or greater and in all exposed groups of females; relative liver weights were significantly increased in 3,121 ppm males (Table G2). The liver weights for exposed groups of males were 109%, 119%, 130%, 137%, and 140% that of the controls for the 3,121, 6,250, 12,500, 25,000, and 50,000 ppm groups, respectively. The liver weights for exposed groups of females were 121%, 117%, 128%, 145%, and 160% that of the controls.

No significant differences in reproductive organ weights, or in sperm parameters and estrous cyclicity were observed between exposed and control groups of male or female rats (Tables H1 and H2).

Incidences of hepatocyte hypertrophy were increased in males and females exposed to 6,250 ppm and were significantly increased in the groups exposed to 12,500 ppm or greater (Table 5). The incidences of cytoplasmic vacuolization of hepatocytes were significantly increased in all exposed groups of males. Hypertrophy was characterized by enlargement of the hepatocytes in the centrilobular areas of the lobules and extended to the midzonal area. Cytoplasmic vacuolization consisted of randomly distributed vacuolated hepatocytes that consisted of either solitary or multiple vacuoles within the cytoplasm of hepatocytes. These vacuoles appeared to have contained fat and were characterized by clear spaces that often displaced the nucleus to the periphery of the cell.

Exposure Concentration Selection Rationale: In the 3-month study, all animals survived, and the final (last in-life) mean body weights of exposed groups were within

10% that of the controls for the 25,000 and 50,000 ppm groups. The severities of hepatocyte centrilobular hypertrophy and increases in liver weights at 25,000 and 50,000 ppm were not considered severe enough to compromise a 2-year study. The type, incidences, and severities of hepatocellular lesions at the 25,000 and 50,000 ppm groups were, in general, similar. The exposure concentrations selected for the 2-year study were 3,000, 9,000, and 25,000 ppm to allow for a broad exposure range.

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 3). Survival of 9,000 ppm females was significantly greater than that of the controls.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of exposed groups of males were similar to those of the control group throughout the study (Figure 4 and Table 7). Mean body weights of females exposed to 9,000 ppm were less than those of the controls after week 37, and those of 25,000 ppm females were less than those of the controls after week 8 (Figure 4 and Table 8). Feed consumption by exposed groups of males and females was generally similar to that by the controls throughout the study (Tables J1 and J2). Dietary concentrations of 3,000, 9,000, and 25,000 ppm resulted in average daily doses of approximately 135, 400, and 1,175 mg/kg for males and 150, 470, and 1,340 mg/kg for females. No clinical findings related to goldenseal root powder exposure were observed.

Table 4
Survival, Body Weights, and Feed Consumption of Rats in the 3-Month Feed Study of Goldenseal Root Powder

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	106 ± 1	324 ± 7	218 ± 3		16.5	17.5
3,121	10/10	106 ± 1	330 ±10	224 ± 10	102	16.8	17.2
6,250	10/10	106 ± 1	336 ±12	230 ± 11	104	16.4	18.8
12,500	10/10	106 ± 1	338 ± 6	233 ± 5	105	16.6	18.5
25,000	10/10	106 ± 2	327 ± 5	222 ± 6	101	15.6	17.5
50,000	10/10	106 ± 1	299 ± 7	193 ± 6	92	14.2	15.2
Female							
0	10/10	89 ± 1	177 ± 4	89 ± 4		12.0	10.5
3,121	10/10	88 ± 1	183 ± 5	95 ± 5	103	12.8	11.1
6,250	10/10	89 ± 1	175 ± 7	85 ± 6	98	11.9	10.6
12,500	10/10	88 ± 1	173 ± 2	84 ± 2	98	12.5	10.3
25,000	10/10	90 ± 1	174 ± 3	84 ± 2	98	12.8	10.6
50,000	10/10	89 ± 1	167 ± 3	78 ± 3	96	11.7	10.3

[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.

TABLE 5
Incidences of Nonneoplastic Lesions of the Liver in Rats in the 3-Month Feed Study of Goldenseal Root Powder

	0 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
Number Examined Microscopically	10	10	10	10	10	10
Hepatocyte, Hypertrophy[a]	0	0	2 (1.0)[b]	9** (1.0)	10** (1.3)	10** (1.8)
Hepatocyte, Vacuolization, Cytoplasmic	1 (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Hepatocyte, Hypertrophy	0	0	3 (1.0)	10** (1.0)	10** (1.9)	10** (2.0)

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

TABLE 6
Survival of Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	12	19	11	24
Natural deaths	5	2	9	1
Animals surviving to study termination	33	29	30	25[a]
Percent probability of survival at end of study[b]	66	58	60	50
Mean survival (days)[c]	679	675	687	663
Survival analysis[d]	P=0.186	P=0.574	P=0.794	P=0.195
Female				
Animals initially in study	50	50	50	50
Moribund	14	9	5	12
Natural deaths	6	5	3	2
Animals surviving to study termination	30	36	42	36
Percent probability of survival at end of study	60	72	84	72
Mean survival (days)	698	693	696	700
Survival analysis	P=0.425N	P=0.367N	P=0.025N	P=0.283N

[a] Includes one animal that died during the last week of the study
[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 control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by **N**.

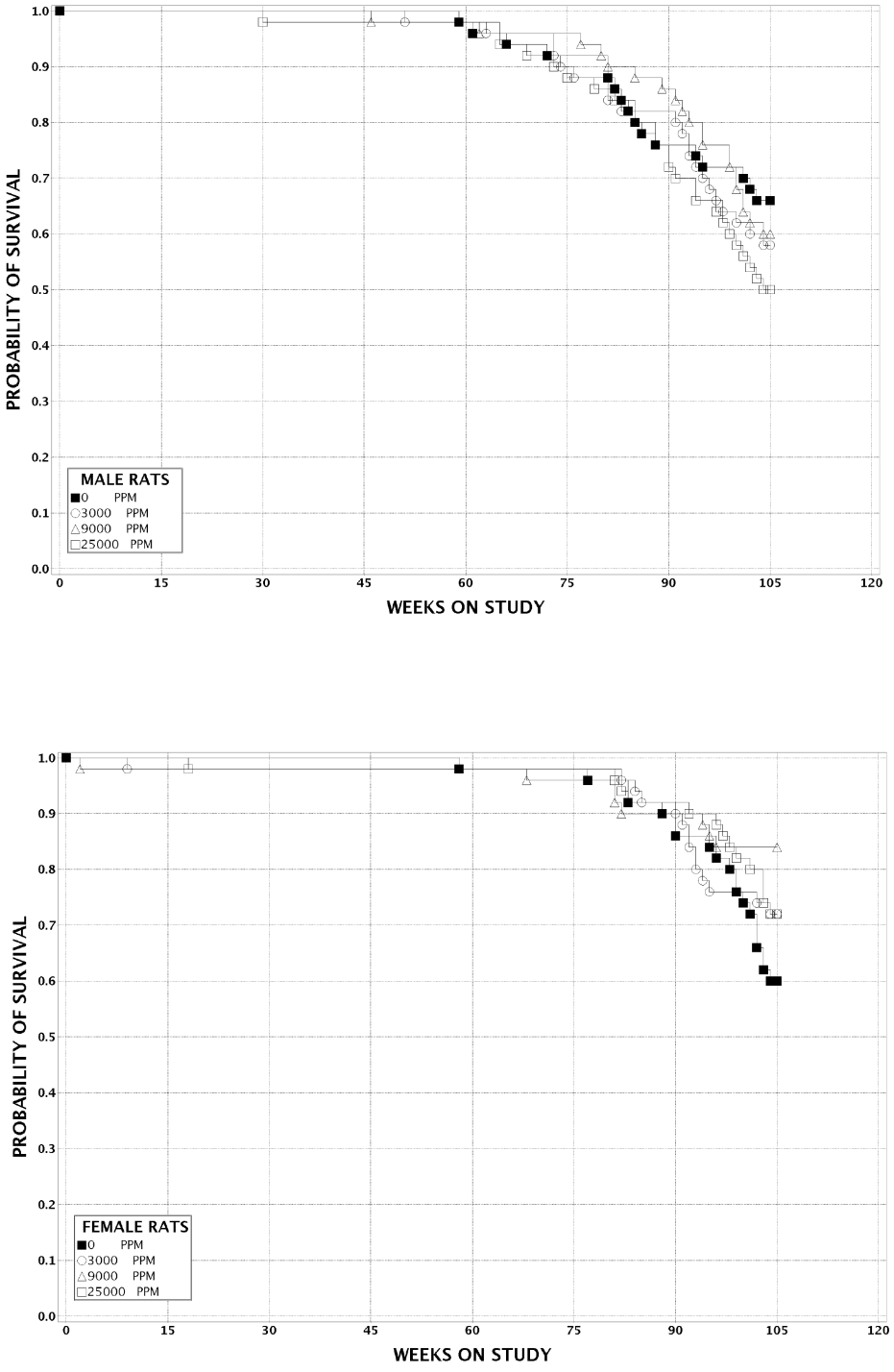


FIGURE 3
Kaplan-Meier Survival Curves for Rats Exposed to Goldenseal Root Powder in Feed for 2 Years

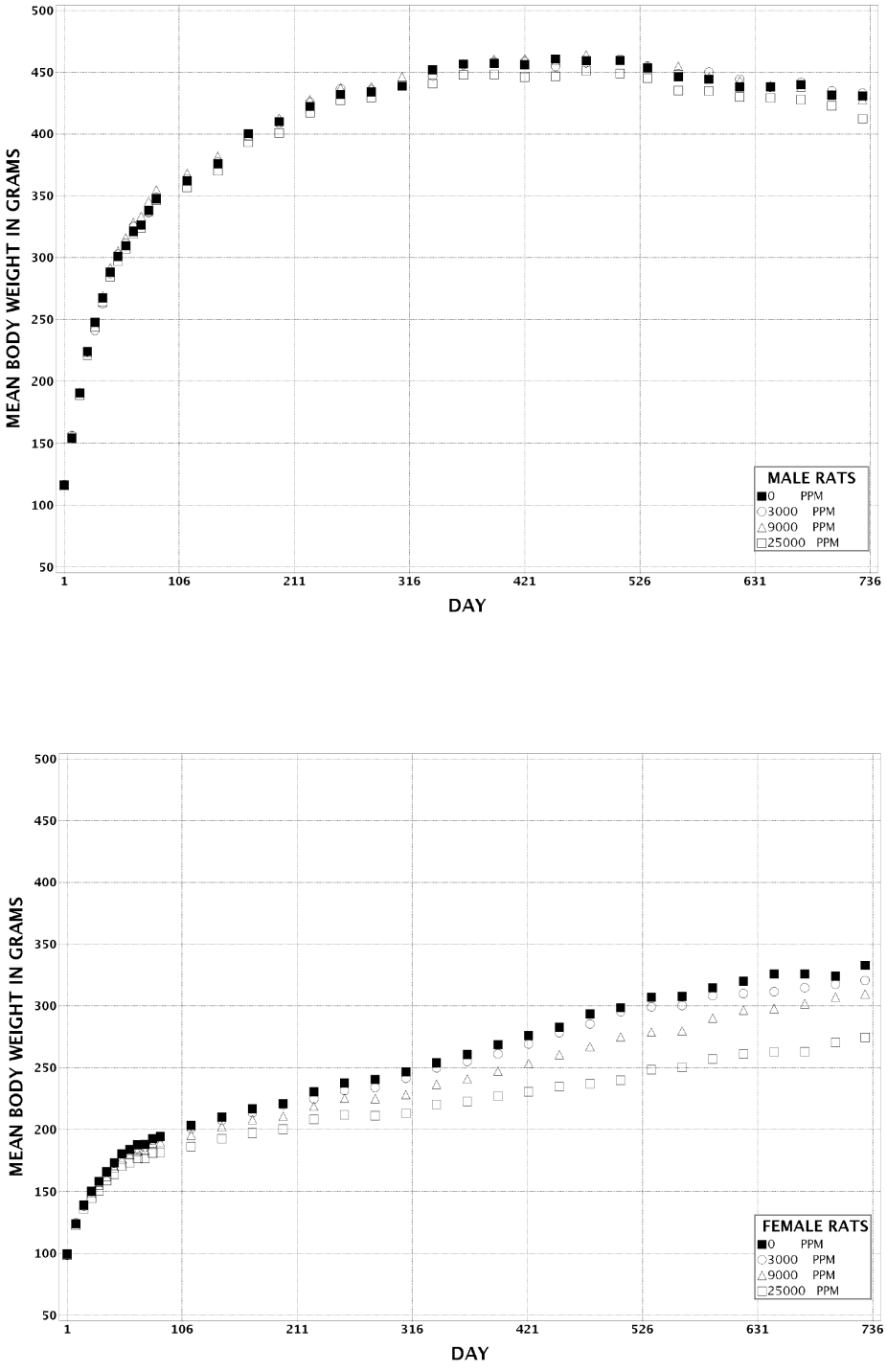


FIGURE 4
Growth Curves for Rats Exposed to Goldenseal Root Powder in Feed for 2 Years

TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

Days on Study	0 ppm		3,000 ppm			9,000 ppm			25,000 ppm		
	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	116	50	117	100	50	117	100	50	116	100	50
8	154	50	157	101	50	156	101	50	155	100	50
15	191	50	191	100	50	192	100	50	189	99	50
22	224	50	224	100	50	223	100	50	221	99	50
29	248	50	241	97	50	247	100	50	244	99	50
36	268	50	263	98	50	269	101	50	264	99	50
43	288	50	287	100	50	292	101	50	284	99	50
50	301	50	303	101	50	305	101	50	297	99	50
57	310	50	312	101	50	316	102	50	307	99	50
64	321	50	325	101	50	328	102	50	319	99	50
71	326	50	326	100	50	333	102	50	324	99	50
78	338	50	337	100	50	346	102	50	337	100	50
85	348	50	349	100	50	355	102	50	347	100	50
113	362	50	364	100	50	368	102	50	357	99	50
141	376	50	377	100	50	382	102	50	370	99	50
169	400	50	399	100	50	399	100	50	393	98	50
197	410	50	409	100	50	413	101	50	401	98	50
225	422	50	426	101	50	428	101	50	417	99	49
253	432	50	437	101	50	437	101	50	427	99	49
281	434	50	436	100	50	438	101	50	430	99	49
309	439	50	439	100	50	446	102	50	439	100	49
337	452	50	447	99	50	452	100	49	441	98	49
365	457	50	456	100	49	457	100	49	448	98	49
393	457	50	459	100	49	460	101	49	448	98	49
421	456	49	459	101	49	461	101	49	446	98	49
449	461	48	455	99	48	460	100	48	447	97	49
477	459	47	458	100	48	464	101	48	451	98	47
508	460	46	461	100	47	460	100	48	449	98	46
533	454	46	455	100	44	455	100	48	445	98	44
561	447	45	449	101	44	455	102	46	435	98	43
589	445	41	450	101	41	447	100	45	435	98	42
617	438	38	444	101	41	443	101	44	430	98	38
645	438	38	438	100	38	439	100	41	430	98	35
673	440	36	442	100	34	438	100	38	428	97	33
701	432	36	435	101	31	432	100	33	423	98	29
Mean for weeks											
1-13	264		264	100		268	101		262	99	
14-52	414		415	100		418	101		408	99	
53-101	450		451	100		452	100		440	98	

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of Goldenseal Root Powder

Days on Study	0 ppm		3,000 ppm			9,000 ppm			25,000 ppm		
	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	100	50	99	99	50	99	99	50	99	99	50
9	124	50	125	101	50	125	101	50	123	99	50
16	139	50	138	99	50	139	100	49	136	98	50
23	150	50	150	100	50	149	99	49	145	96	50
30	158	50	158	100	50	155	98	49	151	95	50
37	166	50	166	100	50	162	98	49	159	96	50
44	173	50	173	100	50	169	98	49	164	95	50
51	181	50	180	100	50	176	98	49	171	95	50
58	184	50	183	100	50	180	98	49	174	94	50
65	188	50	185	99	49	183	97	49	177	94	50
72	188	50	186	99	49	183	97	49	177	94	50
79	193	50	192	99	49	190	98	49	181	94	50
86	195	50	194	100	49	190	98	49	182	94	50
114	203	50	201	99	49	196	97	49	186	92	50
142	210	50	208	99	49	202	96	49	193	92	49
170	217	50	214	99	49	208	96	49	197	91	49
198	221	50	220	100	49	211	96	49	200	91	49
226	231	50	225	98	49	219	95	49	208	90	49
254	238	50	232	98	49	225	95	49	212	89	49
282	240	50	234	97	49	225	94	49	211	88	49
310	247	50	242	98	49	228	93	49	213	86	49
338	254	50	250	98	49	237	93	49	220	87	49
366	261	50	255	98	49	241	92	49	223	85	49
394	269	50	261	97	49	247	92	49	227	85	49
422	276	49	269	98	49	253	92	49	231	84	49
450	283	49	278	98	49	261	92	49	235	83	49
478	294	49	285	97	49	267	91	48	237	81	49
506	299	49	295	99	49	275	92	48	240	80	49
534	307	49	299	97	49	279	91	48	248	81	49
562	308	48	300	98	49	280	91	48	250	81	49
590	315	46	308	98	46	290	92	45	257	82	46
618	320	45	310	97	46	297	93	45	261	82	46
646	326	43	312	96	42	298	91	45	263	81	45
674	326	41	315	97	38	302	93	42	263	81	44
702	324	37	318	98	38	307	95	42	270	83	40
Mean for weeks											
1-13	165		164	100		162	99		157	96	
14-52	229		225	98		217	95		204	90	
53-101	301		293	98		277	92		247	82	

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 liver, pituitary gland, spleen, mammary gland, heart, lung, and nose. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Liver: The incidence of hepatocellular adenoma was significantly increased in males exposed to 25,000 ppm (Tables 9, A1, and A2). The incidence of multiple hepatocellular adenoma was increased, but not significantly, in the 25,000 ppm males. In addition, a hepatocellular carcinoma was observed in one 25,000 ppm male. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in 25,000 ppm males. The incidence of hepatocellular adenoma in 25,000 ppm females was significantly increased (Tables 9, B1, and B2).

The hepatocellular adenomas were typically composed of solid sheets of large eosinophilic, often vacuolated cells with no normal hepatic lobular architecture that resulted in compression of the surrounding nonneoplastic liver parenchyma. Multiple hepatocellular adenomas were seen in two 25,000 ppm males. The hepatocellular carcinoma in one 25,000 ppm male was a large mass composed of large, pleomorphic, markedly vacuolated cells that formed trabeculae often more than five hepatocytes thick.

Increased incidences of nonneoplastic hepatic lesions in exposed groups of males and females included hepatocytic hypertrophy, hepatocytic degeneration, eosinophilic focus, and mixed cell focus (Tables 9, A4, and B4). The incidences generally increased with increasing exposure concentration.

All exposed groups of males and females had significantly increased incidences of hepatocyte hypertrophy, and the severity increased with increasing exposure concentration (Tables 9, A4, and B4). The incidences of hepatocyte degeneration were significantly increased in all exposed groups of males and in 9,000 and 25,000 ppm females, and the severity increased with increasing exposure concentration. Hepatocyte hypertrophy consisted of lobular areas of minimally to moderately enlarged hepatocytes with increased amounts of eosinophilic cytoplasm accompanied by various degrees of vacuolization. Centrilobular and midzonal regions of the liver were typically affected with relative sparing of the periportal areas. In more severely affected livers, however, virtually the entire liver parenchyma was composed of altered hepatocytes. Hepatocytic degeneration was characterized by large hepatocytes distended with either finely granular, pale, slightly eosinophilic cytoplasm or numerous small, discrete clear vacuoles. These microvesicular cytoplasmic changes did not progress to larger clear vacuoles seen with fatty changes. Nuclei were sometimes hyperchromatic and sometimes hypochromatic. The degeneration was accompanied by occasional individual cell necrosis.

The incidences of eosinophilic focus were significantly increased in 9,000 and 25,000 ppm males and all exposed groups of females (Tables 9, A4, and B4). Eosinophilic foci were discrete areas composed of enlarged hepatocytes containing homogenous or finely granular, eosinophilic cytoplasm. Hepatocytes within eosinophilic foci tended to be larger than surrounding hepatocytes, often resulting in some compression of adjacent parenchyma.

The incidence of mixed cell focus was significantly increased in 3,000 ppm males (Tables 9, A4, and B4). Mixed cell foci are composed of two or more kinds of cell types, with no cell type constituting 80% or more of the focus.

The incidences of clear cell focus were significantly decreased in the 9,000 and 25,000 ppm male groups (Tables 9 and A4). The incidence of clear cell focus was increased in 9,000 ppm females but was not significantly different from that in the control group (Tables 9 and B4). The cause for changes in the incidences of clear cell foci in males is unknown. The increased incidence in females appeared unrelated to exposure to goldenseal root powder. Clear cell foci are composed of cells with clear vacuoles in an eosinophilic cytoplasm. The vacuoles contain glycogen and are not typically as discrete as fat-containing vacuoles. The hepatocytes may be larger than normal due to the accumulation of glycogen, and may cause some compression of surrounding parenchyma. Clear cell foci varied in size and shape and often lacked the distinct borders of basophilic and eosinophilic foci.

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Hepatocyte, Hypertrophy[a]	0	19** (1.4)[b]	31** (1.9)	27** (2.6)
Hepatocyte, Degeneration	0	22** (1.1)	30** (1.8)	19** (2.3)
Eosinophilic Focus	4	5	25**	28**
Mixed Cell Focus	9	21**	13	7
Clear Cell Focus	19	17	3**	2**
Basophilic Focus	20	33*	22	13
Hepatocellular Adenoma, Multiple	0	0	0	2
Hepatocellular Adenoma (includes multiple)[c]				
Overall rate[d]	1/50 (2%)	1/50 (2%)	2/50 (4%)	10/50 (20%)
Adjusted rate[e]	2.4%	2.4%	4.6%	24.2%
Terminal rate[f]	1/33 (3%)	0/29 (0%)	1/30 (3%)	5/25 (20%)
First incidence (days)	729 (T)	684	663	612
Poly-3 test[g]	P<0.001	P=0.758	P=0.511	P=0.003
Hepatocellular Carcinoma[h]	0	0	0	1
Hepatocellular Adenoma or Carcinoma[i]				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	11/50 (22%)
Adjusted rate	2.4%	2.4%	4.6%	26.5%
Terminal rate	1/33 (3%)	0/29 (0%)	1/30 (3%)	5/25 (20%)
First incidence (days)	729 (T)	684	663	612
Poly-3 test	P<0.001	P=0.758	P=0.511	P<0.001

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Female				
Number Examined Microscopically	50	50	50	50
Hepatocyte, Hypertrophy	2 (2.0)	10* (1.3)	27** (1.5)	38** (2.1)
Hepatocyte, Degeneration	1 (1.0)	2 (1.0)	12** (1.1)	24** (1.3)
Eosinophilic Focus	2	24**	29**	22**
Mixed Cell Focus	6	5	13	9
Clear Cell Focus	4	5	10	2
Basophilic Focus	44	44	46	27**
Hepatocellular Adenoma[j]				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	8/50 (16%)
Adjusted rate	0.0%	0.0%	2.2%	17.2%
Terminal rate	0/30 (0%)	0/36 (0%)	1/42 (2%)	6/36 (17%)
First incidence (days)	—[k]	—	729 (T)	666
Poly-3 test	P<0.001	—[l]	P=0.505	P=0.004

* Significantly different (P≤0.05) from the control group by the Poly-3 test
** (P≤0.01)
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year feed studies with controls given NTP-2000 diet (mean ± standard deviation): 7/300 (2.3% ± 2.3%), range 0%-6%; all routes: 17/1,399 (1.2% ± 1.7%), range 0%-6%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence by all routes: 1/1,250
[i] Historical incidence for feed studies: 7/300 (2.3% ± 2.3%), range 0%-6%; all routes: 22/1,399 (1.6% ± 1.7%), range 0%-6%
[j] Historical incidence for feed studies: 4/250 (1.6% ± 2.2%), range 0%-4%; all routes: 16/1,350 (1.2% ± 2.6%), range 0%-12%
[k] Not applicable; no neoplasms in animal group
[l] Value of statistic cannot be computed.

The incidence of basophilic focus in females was significantly decreased in 25,000 ppm females (Tables 9 and B4). Incidences of basophilic focus were increased in 3,000 ppm males and decreased, though not significantly, in 25,000 ppm males and appeared unrelated to exposure (Tables 9 and A4). Basophilic foci were composed of hepatocytes that were normal or, more frequently, smaller than normal and had basophilic cytoplasm. The margins were distinct and they had normally arranged hepatic cords. The cytoplasm sometimes had tigroid patterns or stripes of basophilia. There was no or minimal compression of the surrounding parenchyma.

Pituitary Gland: The incidences of adenoma in the pars distalis were increased in all exposed groups of males with significant increases in the 3,000 and 9,000 ppm groups (0 ppm, 8/50; 3,000 ppm, 18/50; 9,000 ppm, 16/49; 25,000 ppm, 13/50; Tables A1 and A2). A significant decrease in the incidences of pars distalis adenoma occurred in 9,000 and 25,000 ppm females (25/50, 20/50, 16/50, 16/50; Tables B1 and B2). The incidences of adenoma or carcinoma (combined) occurred with a negative trend, but incidences in 9,000 and 25,000 ppm females were not significantly different from that in the control group (25/50, 24/50, 18/50, 17/50).

Pituitary gland adenomas consisted of localized proliferation of pituitary cells, primarily chromophobes, with definite compression or displacement of the surrounding normal tissues. They lacked capsules. Most of them were extremely vascular having numerous blood-filled spaces. Some were cystic. The smaller adenomas tended to be solid cellular masses. Some adenomas had considerable cellular pleomorphism and nuclear atypia without evidence of invasion of the adjacent tissues. Carcinomas were similar in appearance to the pleomorphic adenomas but had definitive evidence of infiltration of the brain.

The incidences of focal hyperplasia were significantly decreased in 3,000 ppm males and females [males: 0 ppm, 14/50 (1.8); 3,000 ppm, 6/50 (2.0); 9,000 ppm, 8/49 (1.6); 25,000 ppm, 10/50 (2.0); females: 13/50 (2.4), 4/50 (2.0), 12/50 (2.0), 9/50 (1.8); Tables A4 and B4]. Hyperplasia of the pituitary gland consisted of ill-defined foci of cell proliferation without nodularity, nuclear atypia, or compression of adjacent tissues. Normal appearing pituitary cells were often scattered within these hyperplastic foci.

The occurrence of pituitary gland adenomas in males in the 9,000 and 25,000 ppm groups was not considered exposure-related. There was no supportive evidence for an increase in preneoplastic pituitary gland lesions, the rate for pituitary gland adenoma and carcinoma was not increased, and the pituitary gland lesion rates for the exposed groups were within the historical control ranges. There was no evidence for an increase in pituitary gland lesions in females.

Spleen: The incidences of hematopoietic cell proliferation were significantly increased in 3,000 and 25,000 ppm males (0 ppm, 2/49; 3,000 ppm, 8/50; 9,000 ppm, 4/49; 25,000 ppm, 11/50; Table A4). Hematopoietic cell proliferation was characterized by an increase in small, deeply basophilic erythroid cells and less often by large immature myeloid cells within the red pulp.

Mammary Gland: The incidences of fibroadenoma occurred with a negative trend and were significantly decreased in all exposed groups of females (Tables 10, B1, and B2). The incidence of hyperplasia was significantly decreased in 25,000 ppm females (Tables 10 and B4).

Fibroadenomas were benign lesions that consisted of proliferating neoplastic fibrous and glandular tissues. They retained the overall histological appearance of normal mammary glands having well-defined lobules of increased glandular tissue separated by various amounts of dense collagenous tissue. The neoplastic epithelial cells were generally uniform in size and shape and arranged in clusters or acinar groups. They had round or oval nuclei and few mitotic figures. The cytoplasm was eosinophilic with vacuolation ranging from small, fine vacuoles and a foamy appearance to coarse larger vacuoles. In some areas, the neoplastic epithelium formed ductular structures having single layers of columnar or cuboidal cells.

Mammary gland hyperplasia resulted in enlarged lobules due to increased amounts of alveolar or ductular epithelial tissues. The epithelium was well differentiated, and the alveoli and ducts had a single layer of cells with no atypia. The size of the epithelial cells varied with the degree of secretory activity and number of lipid vacuoles. Connective tissue stroma was scant.

Heart: The incidences of cardiomyopathy were significantly decreased in all exposed groups of males and in 25,000 ppm females (males: 0 ppm, 47/50; 3,000 ppm, 38/50; 9,000 ppm, 39/50; 25,000 ppm 36/50, females: 24/50, 24/50, 21/50, 15/50, Tables A4 and B4). The severities of this lesion were generally similar.

Cardiomyopathy consisted of several microscopic changes in localized, often multiple areas of the myocardium including the loss of myocytes, coagulative necrosis and mineralization of small numbers of individual myocytes, limited inflammatory cell infiltrates composed of a few macrophages and lymphocytes with occasional neutrophils, and various degrees of fibrosis.

Other Nonneoplastic Lesions: Incidences of chronic lung inflammation were significantly decreased in 25,000 ppm males (0 ppm, 16/50; 3,000 ppm, 10/50; 9,000 ppm, 17/50; 25,000 ppm, 3/50; Table A4), and the lungs of all exposed groups of males had significant decreases in the incidences of histiocytic cellular infiltrates (34/50, 24/50, 25/50, 24/50). Infiltrates in the alveolar, peribronchiolar, or peribronchial tissues of the lungs consisting of mixed cell types such as neutrophils, macrophages, and lymphocytes, were termed chronic inflammation.

The incidences of chronic nasal inflammation were significantly decreased in all exposed groups of males (12/50, 4/50, 3/50, 3/50; Table A4). When the nasal tissues had infiltrates composed of a variety of cell types, including neutrophils, macrophages, and lymphocytes, accompanied by some degree of fibrosis in the submucosal tissues, the lesion was termed chronic inflammation.

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Mammary Gland in Female Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Number Necropsied	50	50	50	50
Hyperplasia[a]	49 (2.5)	46 (2.4)[b]	45 (2.5)	34** (2.6)
Fibroadenoma, Multiple	8	3	4	1**
Fibrodenoma (includes multiple)[c]				
Overall rate[d]	30/50 (60%)	20/50 (40%)	11/50 (22%)	17/50 (34%)
Adjusted rate[e]	64.6%	43.5%	24.0%	36.2%
Terminal rate[f]	21/30 (70%)	17/36 (47%)	11/42 (26%)	13/36 (36%)
First incidence (days)	611	585	729 (T)	568
Poly-3 test[g]	P=0.015N	P=0.029N	P=0.001N	P=0.004N

** Significantly different (P≤0.01) from the control group by the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year feed studies with controls given NTP-2000 diet (mean ± standard deviation): 138/250 (55.2% ± 4.6%), range 48%-60%; all routes: 697/1,350 (51.6% ± 14.9%), range 24%-86%
[d] Number of animals with neoplasm per number of animals with mammary gland necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.

MICE

2-WEEK STUDY

All mice survived to the end of the study (Table 11). Final mean body weights and body weight gains of exposed animals were similar to those of the controls. Feed consumption by exposed groups was similar to that by the controls. Dietary concentrations of 1,560, 3,121, 6,250, 12,500, 25,000, and 50,000 ppm resulted in average daily doses of approximately 380, 840, 1,760, 3,435, 6,700, and 15,170 mg goldenseal root powder/kg body weight for males and 330, 670, 1,240, 2,375, 4,760, and 8,475 mg/kg for females. No clinical findings related to goldenseal root powder exposure were observed.

Significant increases in absolute and relative liver weights occurred in males exposed to 25,000 or 50,000 ppm and in females exposed to 50,000 ppm (Table G3). The liver weights of exposed groups of males were 99%, 102%, 105%, 105%, 123%, and 136% that of the controls for the 1,560, 3,121, 6,250, 12,500, 25,000, and 50,000 ppm groups, respectively. The liver weights of exposed groups of females were 100%, 92%, 108%, 96%, 101%, and 119% that of the controls. Absolute and relative thymus weights of 12,500 and 50,000 ppm males were significantly decreased.

Minimal hypertrophy of centrilobular hepatocytes was observed in all males and females exposed to 50,000 ppm [males: 0/5; 0/0; 0/0; 0/0; 0/0; 0/5; 5/5 (1.0); females: 0/5, 0/0, 0/0, 0/0, 0/0, 0/5, 5/5 (1.0)]. This change was not observed in any 25,000 ppm males or females.

Exposure Concentration Selection Rationale: In the 2-week study, all animals survived, and the final mean body weights of the 50,000 ppm groups were within 7% those of the controls. The severity of hepatocyte centrilobular hypertrophy and increases in liver weights in the exposed groups were not considered severe enough to compromise a 3-month study. The exposure concentrations selected for the 3-month study were 3,121, 6,250, 25,000, and 50,000 ppm.

TABLE 11
Survival, Body Weights, and Feed Consumption of Mice in the 2-Week Feed Study of Goldenseal Root Powder

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	19.6 ± 0.7	22.5 ± 0.4	2.8 ± 0.4		5.2	6.5
1,560	5/5	20.2 ± 0.4	22.3 ± 0.4	2.1 ± 0.5	99	5.1	5.7
3,121	5/5	20.8 ± 0.4	22.7 ± 0.5	1.9 ± 0.2	101	5.3	6.8
6,250	5/5	19.9 ± 0.4	22.3 ± 0.2	2.4 ± 0.5	99	4.9	7.3
12,500	5/5	19.9 ± 0.2	21.7 ± 0.4	1.8 ± 0.5	96	5.1	6.7
25,000	5/5	20.0 ± 0.3	22.5 ± 0.2	2.5 ± 0.2	100	5.0	6.8
50,000	5/5	18.7 ± 0.8	20.9 ± 0.6	2.2 ± 0.5	93	5.0	7.5
Female							
0	5/5	18.0 ± 0.5	19.4 ± 0.4	1.4 ± 0.2		3.1	3.8
1,560	5/5	18.0 ± 0.3	19.4 ± 0.4	1.4 ± 0.4	100	3.8	4.0
3,121	5/5	18.1 ± 0.6	19.2 ± 0.4	1.1 ± 0.2	99	3.5	4.3
6,250	5/5	17.6 ± 0.4	20.0 ± 0.3	2.4 ± 0.2	103	3.8	3.5
12,500	5/5	17.5 ± 0.4	19.2 ± 0.2	1.7 ± 0.3	99	3.3	3.6
25,000	5/5	17.8 ± 0.5	19.3 ± 0.6	1.5 ± 0.3	99	3.3	3.7
50,000	5/5	17.8 ± 0.4	19.2 ± 0.3	1.4 ± 0.1	99	2.6	3.6

[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the control group are not significant by Dunnett's test.
[c] Feed consumption is expressed as grams per animal per day.

3-Month Study

All mice survived to the end of the study (Table 12). Final (last in-life) mean body weights of males exposed to 50,000 ppm and final mean body weights and mean body weight gains of females exposed to 25,000 or 50,000 ppm were significantly less than those of the controls. Feed consumption by 3,121, 6,250, 12,500, 25,000, and 50,000 ppm males was generally similar to that by the controls. Feed consumption by exposed groups of females was generally similar to that by the controls. Dietary concentrations of 3,121, 6,250, 12,500, 25,000, and 50,000 ppm resulted in average daily doses of approximately 680, 1,360, 2,260, 5,370, and 10,550 mg/kg for males and 590, 1,250, 2,345, 4,790, and 10,740 mg/kg for females. No clinical findings related to goldenseal root powder exposure were observed.

No changes in hematology variables were attributable to goldenseal root powder administration (Table F2).

Absolute and relative liver weights were significantly increased relative to controls in males exposed to 12,500 ppm or greater and in females exposed to 25,000 or 50,000 ppm (Table G4). The liver weights of exposed groups of males were 104%, 106%, 114%, 122%, and 143% that of the controls for the 0, 3,121, 6,250, 12,500, 25,000, and 50,000 ppm groups, respectively. The liver weights of exposed groups of females were 99%, 103%, 99%, 111%, and 111% that of the controls.

The absolute epididymal weight for male mice was significantly decreased relative to the controls (Table H3); however, no significant differences in reproductive parameters were observed between exposed and control groups of males or females (Table H4).

Table 12
Survival, Body Weights, and Feed Consumption of Mice in the 3-Month Feed Study of Goldenseal Root Powder

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	21.4 ± 0.3	32.4 ± 0.5	11.0 ± 0.5		6.1	5.7
3,121	10/10	21.2 ± 0.4	31.7 ± 0.8	10.5 ± 0.6	98	5.3	5.5
6,250	10/10	21.6 ± 0.2	32.0 ± 0.5	10.3 ± 0.4	99	5.3	5.7
12,500	10/10	21.3 ± 0.4	32.8 ± 0.6	11.5 ± 0.4	101	5.3	5.6
25,000	10/10	21.2 ± 0.4	31.6 ± 0.5	10.5 ± 0.5	98	6.2	6.0
50,000	10/10	20.6 ± 0.4	29.9 ± 0.4*	9.3 ± 0.5	92	5.5	5.5
Female							
0	10/10	18.2 ± 0.4	26.9 ± 0.7	8.7 ± 0.5		3.4	4.9
3,121	10/10	17.9 ± 0.4	27.3 ± 0.7	9.5 ± 0.5	102	2.9	4.8
6,250	10/10	17.6 ± 0.4	27.6 ± 0.7	10.0 ± 0.4	103	3.4	5.4
12,500	10/10	18.2 ± 0.2	26.3 ± 0.3	8.0 ± 0.2	98	2.8	5.0
25,000	10/10	18.1 ± 0.2	25.1 ± 0.2*	7.0 ± 0.3**	93	2.8	4.5
50,000	10/10	17.9 ± 0.3	22.9 ± 0.5**	5.0 ± 0.4**	85	3.2	4.3

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** ($P \leq 0.01$)
[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.

The increased incidences of hepatocyte hypertrophy in males and females exposed to 12,500 ppm or greater were consistent with increased liver weights (Table 13). The increased incidences of glycogen depletion in the liver of 50,000 ppm males and females exposed to 12,500 ppm or greater were considered exposure-related.

Exposure Concentration Selection Rationale: In the 3-month study, all animals survived. The final mean body weight of 50,000 ppm females was decreased. The hepatocyte centrilobular hypertrophy in the 50,000 ppm groups was somewhat more severe than in the 25,000 ppm groups. The concentrations selected for the 2-year study were 3,000, 9,000, and 25,000 ppm to allow for a broad range of exposure.

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 14 and in the Kaplan-Meier survival curves (Figure 5). Survival of 9,000 ppm female mice was significantly less than that of the controls.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of all exposed groups of males and females exposed to 3,000 or 9,000 ppm were similar to those of the control groups throughout the study (Tables 15 and 16; Figure 6). Mean body weights of females exposed to 25,000 ppm were generally less than those of the controls after week 13. Feed consumption by exposed groups of males and females was generally similar to that by the controls throughout the study (Tables J3 and J4). Dietary concentrations of 3,000, 9,000, and 25,000 ppm resulted in average daily doses of approximately 375, 1,120, and 3,275 mg/kg body weight for males and 330, 1,000, and 2,875 mg/kg for females. No clinical findings related to goldenseal root powder exposure were observed.

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 liver. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms

Table 13
Incidences of Nonneoplastic Lesions of the Liver in Mice in the 3-Month Feed Study of Goldenseal Root Powder

	0 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
Number Examined Microscopically	10	10	10	10	10	10
Depletion, Glycogen[a]	5 (1.0)[b]	5 (1.4)	4 (1.0)	1 (1.0)	2 (1.0)	10* (1.6)
Hepatocyte, Hypertrophy	0	0	0	4* (1.0)	10**(1.9)	10**(2.3)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Depletion, Glycogen	0	1 (1.0)	0	7**(1.9)	10**(2.2)	10**(2.1)
Hepatocyte, Hypertrophy	0	0	0	9**(1.0)	10**(2.3)	10**(2.6)

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

that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: Increased incidences of hepatoblastoma occurred with a positive trend in males with a marginal increase in the 25,000 ppm group (Tables 17, C1, and C2). Multiple hepatoblastomas were seen in two 25,000 ppm males. The incidences of hepatocellular adenoma occurred with a positive trend in males with a slight increase in the 25,000 ppm group. The incidences of multiple hepatocellular adenoma were significantly increased in 9,000 and 25,000 ppm males. The incidences of hepatocellular carcinoma were increased, but not significantly, in all exposed groups of males. Small and not significant increases in the incidences of hepatocellular adenoma occurred in all exposed groups of females (Tables 17, D1, and D2).

Microscopically, the hepatoblastomas were usually well-demarcated from the surrounding tissue. They had a distinctive appearance consisting of nests, clusters, or sheets of small to medium sized, generally spindle-shaped cells that had scant amounts of deeply basophilic cytoplasm and round to irregular hyperchromatic nuclei. Hepatoblastomas sometimes occurred within a hepatocellular carcinoma and at other times appeared to arise directly from the liver parenchyma. Hepatoblastomas are malignant neoplasms that are presumed to be a primitive form of hepatocellular carcinoma.

TABLE 14
Survival of Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	0	3	2	3
Natural deaths	7	9	3	2
Animals surviving to study termination	43	38[a]	45	45
Percent probability of survival at end of study[b]	86	76	90	90
Mean survival (days)[c]	715	708	715	723
Survival analysis[d]	P=0.245N	P=0.310	P=0.771N	P=0.716N
Female				
Animals initially in study	50	50	50	50
Moribund	4	2	2	3
Natural deaths	1	4	12	4
Animals surviving to study termination	45[a]	44	36	43
Percent probability of survival at end of study	90	88	72	86
Mean survival (days)	716	712	691	707
Survival analysis	P=0.744	P=0.985	P=0.046	P=0.744

[a] Includes one animal that died during the last week of the study
[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 control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by **N**.

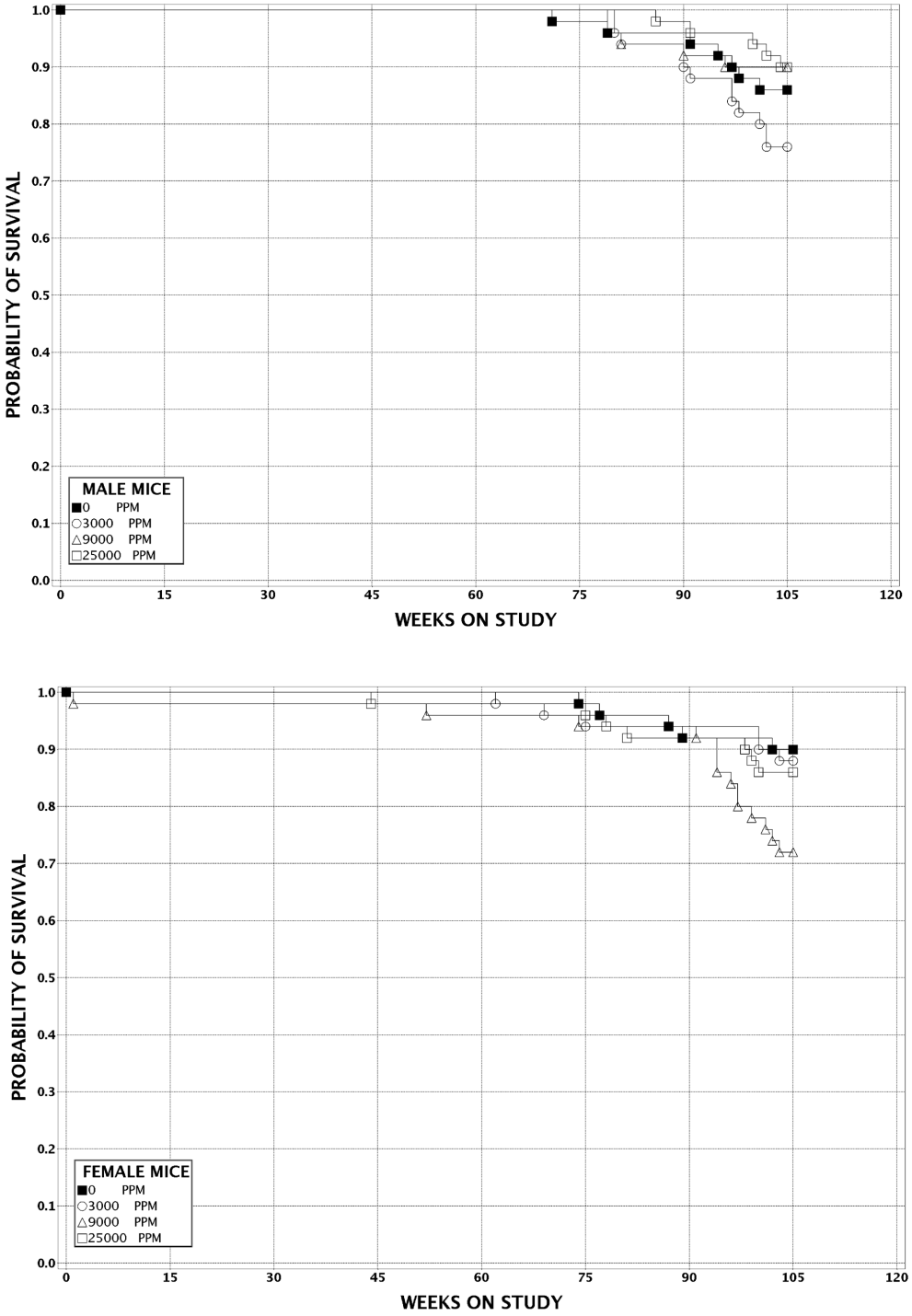


FIGURE 5
Kaplan-Meier Survival Curves for Mice Exposed to Goldenseal Root Powder in Feed for 2 Years

TABLE 15
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of Goldenseal Root Powder

Days on Study	0 ppm		3,000 ppm			9,000 ppm			25,000 ppm		
	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	20.4	50	20.5	100	50	20.7	101	50	20.6	101	50
8	21.1	50	21.6	102	50	21.7	103	50	21.2	101	50
15	22.8	50	22.9	100	50	23.1	101	50	22.9	100	50
22	23.8	50	24.2	102	50	24.1	101	50	24.1	101	50
29	24.5	50	25.1	102	50	24.4	100	50	24.8	101	50
36	25.5	50	25.8	101	50	25.6	101	50	25.6	101	50
43	26.4	50	27.1	102	50	26.6	101	50	26.6	101	50
50	27.1	50	27.8	103	50	27.1	100	50	27.2	100	50
57	28.2	50	29.2	104	50	28.6	101	50	28.5	101	50
64	29.2	50	30.1	103	50	28.7	98	50	28.8	99	50
71	30.4	50	31.0	102	50	30.0	99	50	29.7	98	50
78	31.0	50	31.8	102	50	30.8	99	50	30.4	98	50
85	32.3	50	32.9	102	50	32.1	99	50	31.3	97	50
113	34.6	50	35.4	102	50	34.7	100	50	33.1	96	50
141	37.7	50	37.0	98	50	36.0	95	50	34.8	92	50
169	36.6	50	37.5	103	50	36.5	100	50	34.9	96	50
197	38.5	50	41.1	107	50	39.7	103	50	38.0	99	50
225	40.6	50	42.4	104	50	41.1	101	50	39.4	97	50
256	43.9	50	44.9	102	50	43.9	100	50	42.1	96	50
281	44.3	50	45.5	103	50	44.3	100	50	42.6	96	50
309	47.3	50	47.9	101	50	47.0	100	50	45.6	97	50
337	48.2	50	48.7	101	50	47.9	99	50	46.7	97	50
365	47.8	50	48.3	101	50	48.2	101	50	47.0	98	50
393	48.1	50	48.8	102	50	48.5	101	50	47.2	98	50
422	47.3	50	48.2	102	50	47.6	101	50	46.7	99	50
449	48.3	50	49.2	102	50	48.2	100	50	46.9	97	50
477	47.8	50	49.0	103	50	47.6	100	50	46.8	98	50
505	47.7	49	48.6	102	50	47.7	100	50	46.4	97	50
533	47.7	49	48.2	101	50	46.9	98	50	45.8	96	50
561	47.4	48	47.8	101	48	47.3	100	48	46.0	97	50
589	46.9	48	48.1	103	47	47.1	101	47	45.7	98	50
618	46.4	48	47.8	103	47	46.0	99	47	45.0	97	49
645	46.3	47	47.0	101	44	46.3	100	46	43.9	95	48
673	45.8	46	45.9	100	43	46.7	102	45	44.0	96	48
701	46.6	44	45.6	98	41	46.3	100	45	44.1	95	47
Mean for weeks											
1-13	26.4		26.9	102		26.4	100		26.3	100	
14-52	41.3		42.3	102		41.2	100		39.7	96	
53-101	47.2		47.9	101		47.3	100		45.8	97	

TABLE 16
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of Goldenseal Root Powder

Days on Study	0 ppm		3,000 ppm			9,000 ppm			25,000 ppm		
	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	15.4	50	15.4	100	50	15.4	101	50	15.2	99	50
9	18.5	50	18.5	100	50	18.0	98	49	18.3	99	50
16	19.1	50	19.2	100	50	19.1	100	49	19.0	100	50
23	20.0	50	20.5	103	50	20.4	102	49	20.2	101	50
30	21.6	50	21.7	101	50	21.3	99	49	21.3	99	50
37	21.5	50	22.2	103	50	21.7	101	49	21.4	99	50
44	23.2	50	23.4	101	50	23.3	101	49	22.7	98	50
51	24.0	50	24.0	100	50	24.1	100	49	22.9	96	50
58	25.0	50	25.1	101	50	24.9	100	49	24.2	97	50
65	24.3	50	24.8	102	50	24.1	99	49	23.2	95	50
72	26.4	50	26.4	100	50	26.1	99	49	25.4	97	50
79	27.3	50	27.0	99	50	26.7	98	49	25.6	94	50
86	28.0	50	28.1	100	50	27.9	100	49	26.6	95	50
114	31.0	50	31.0	100	50	30.1	97	49	29.0	93	50
142	33.0	50	32.9	100	50	32.5	98	49	30.1	91	50
170	31.9	50	33.0	104	50	32.1	100	49	30.3	95	50
198	33.7	50	36.4	108	50	34.8	103	49	32.8	97	50
226	35.7	50	38.3	107	50	36.6	103	49	33.9	95	50
254	38.4	50	39.9	104	50	38.6	101	49	35.9	94	50
282	41.0	50	42.6	104	50	41.5	101	49	38.8	95	50
310	44.7	50	46.0	103	50	45.1	101	49	41.5	93	49
338	47.1	50	47.9	102	50	47.5	101	49	44.3	94	49
366	47.0	50	48.7	104	50	48.2	103	48	44.8	95	49
394	47.2	50	50.0	106	50	48.7	103	48	46.0	97	49
422	47.4	50	49.4	104	50	49.0	104	48	45.9	97	49
450	49.3	50	51.5	105	49	51.2	104	48	47.3	96	49
478	50.0	50	51.3	103	49	51.1	102	48	46.3	93	49
506	50.1	50	52.1	104	48	51.4	103	48	47.0	94	49
534	50.4	49	52.5	104	47	52.1	104	47	47.7	95	48
562	50.7	48	52.5	104	47	52.2	103	47	47.6	94	47
590	50.8	48	52.8	104	47	52.9	104	47	48.5	96	46
618	50.7	47	53.6	106	47	53.3	105	47	48.3	95	46
646	51.8	46	54.2	105	47	52.7	102	46	48.8	94	46
674	51.3	46	53.9	105	47	53.2	104	42	48.3	94	46
702	51.9	46	54.4	105	45	53.7	104	39	49.2	95	43
Mean for weeks											
1-13	22.6		22.8	101		22.5	100		22.0	98	
14-52	37.4		38.7	103		37.6	101		35.2	94	
53-101	49.9		52.1	104		51.5	103		47.4	95	

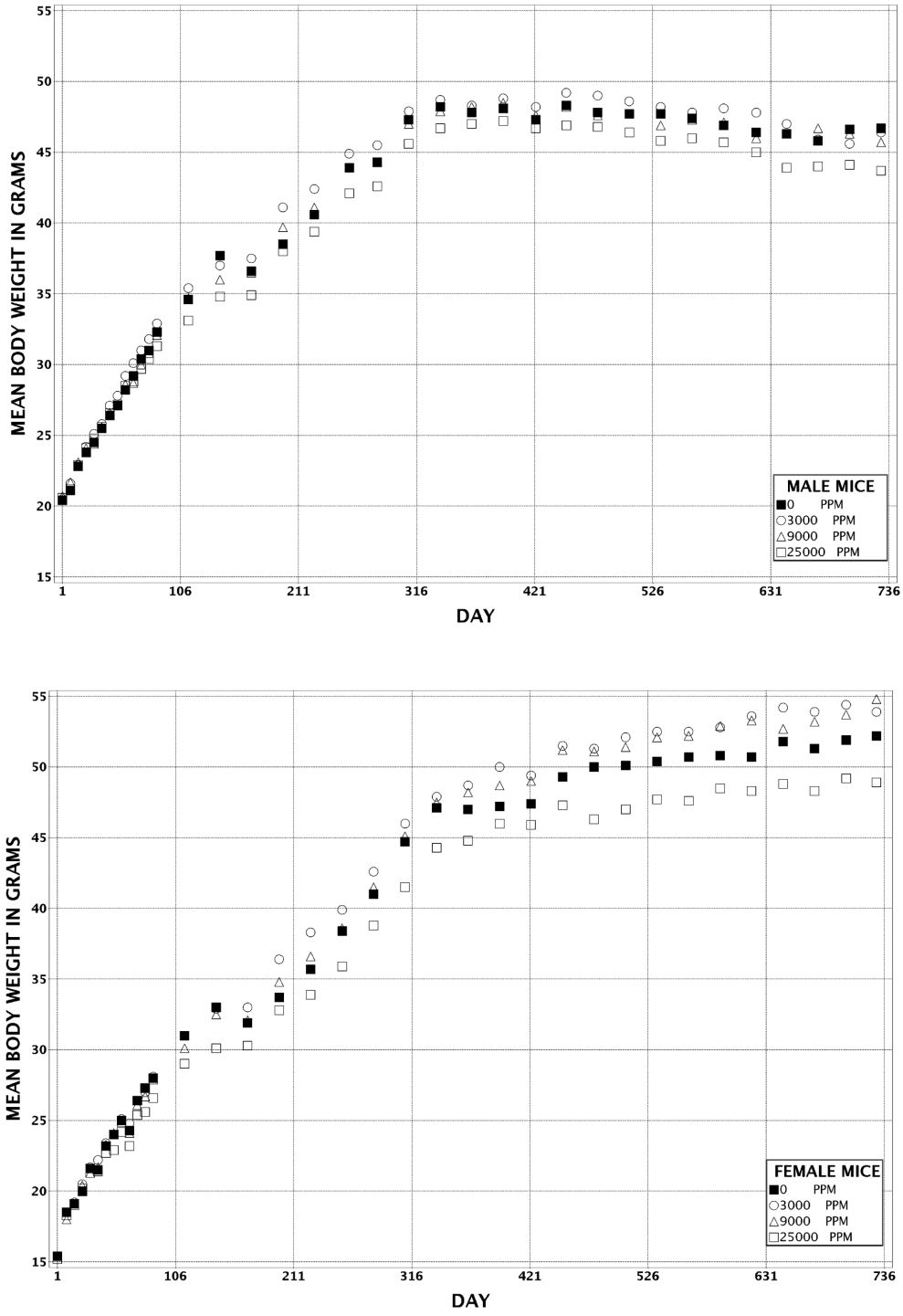


FIGURE 6
Growth Curves for Mice Exposed to Goldenseal Root Powder in Feed for 2 Years

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Eosinophilic Focus[a]	7	14	14	24**
Mixed Cell Focus	1	7*	9**	6
Hepatoblastoma, Multiple	0	0	0	2
Hepatoblastoma (includes multiple)[b]				
Overall rate[c]	1/50 (2%)	2/50 (4%)	1/50 (2%)	6/50 (12%)
Adjusted rate[d]	2.1%	4.3%	2.1%	12.3%
Terminal rate[e]	1/43 (2%)	1/38 (3%)	1/45 (2%)	5/45 (11%)
First incidence (days)	729 (T)	673	729 (T)	722
Poly-3 test[f]	P=0.016	P=0.492	P=0.760N	P=0.061
Hepatocellular Adenoma, Multiple	3	5	11*	18**
Hepatocellular Adenoma (includes multiple)[g]				
Overall rate	22/50 (44%)	16/50 (32%)	23/50 (46%)	29/50 (58%)
Adjusted rate	45.7%	33.8%	48.0%	58.2%
Terminal rate	19/43 (44%)	13/38 (34%)	22/45 (49%)	26/45 (58%)
First incidence (days)	677	555	666	596
Poly-3 test	P=0.030	P=0.162N	P=0.492	P=0.150
Hepatocellular Carcinoma, Multiple	2	4	4	2
Hepatocellular Carcinoma (includes multiple)[h]				
Overall rate	8/50 (16%)	14/50 (28%)	15/50 (30%)	12/50 (24%)
Adjusted rate	16.7%	28.7%	30.3%	24.2%
Terminal rate	6/43 (14%)	8/38 (21%)	11/45 (24%)	8/45 (18%)
First incidence (days)	681	555	551	596
Poly-3 test	P=0.436	P=0.120	P=0.088	P=0.254
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[i]				
Overall rate	26/50 (52%)	28/50 (56%)	31/50 (62%)	33/50 (66%)
Adjusted rate	54.0%	56.7%	62.7%	66.0%
Terminal rate	23/43 (54%)	19/38 (50%)	27/45 (60%)	28/45 (62%)
First incidence (days)	677	555	551	596
Poly-3 test	P=0.128	P=0.473	P=0.252	P=0.156

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Female				
Number Examined Microscopically	50	50	49	50
Hepatocellular Adenoma, Multiple	0	0	1	2
Hepatocellular Adenoma (includes multiple)[j]				
Overall rate	3/50 (6%)	6/50 (12%)	7/49 (14%)	7/50 (14%)
Adjusted rate	6.3%	12.6%	15.6%	14.9%
Terminal rate	3/45 (7%)	6/44 (14%)	6/46 (17%)	7/43 (16%)
First incidence (days)	729 (T)	729 (T)	654	729 (T)
Poly-3 test	P=0.197	P=0.239	P=0.132	P=0.149

* Significantly different (P≤0.05) from the control group by the Poly-3 test
** (P≤0.01)
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Historical incidence for 2-year feed studies with controls given NTP-2000 diet (mean ± standard deviation): 1/250 (0.4% ± 0.9%), range 0%-2%; all routes: 48/1,447 (3.3% ± 6.4%), range 0%-34%
[c] Number of animals with neoplasm per number of animals with liver examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposed group is indicated by **N**.
[g] Historical incidence for feed studies: 83/250 (33.2% ± 10.1%), range 22%-44%; all routes: 733/1,447 (50.7% ± 13.9%), range 22%-72%
[h] Historical incidence for feed studies: 58/250 (23.2% ± 8.3%), range 16%-34%; all routes: 415/1,447 (28.7% ± 8.8%), range 16%-52%
[i] Historical incidence for feed studies: 125/250 (50.0% ± 10.2%), range 36%-64%; all routes: 972/1,447 (67.2% ± 13.1%), range 36%-92%
[j] Historical incidence for feed studies: 20/300 (6.7% ± 2.7%), range 2%-10%; all routes: 396/1,494 (26.5% ± 15.2%), range 2%-54%

Many of the hepatocellular adenomas had the typical appearance of those reported in B6C3F1 mice. These were discrete masses with distinct borders that caused compression of the surrounding normal hepatic parenchyma, sometimes projecting above the surface of the liver. They were composed of mildly to moderately pleomorphic hepatocytes that were of normal size or slightly larger than normal. Neoplastic hepatocytes within the adenomas frequently resembled those seen in eosinophilic foci but basophilic, clear, and vacuolated hepatocytes were also present. The normal lobular architecture was disrupted when plates of neoplastic hepatocytes intersected the surrounding normal hepatocytes at oblique or right angles, making the adenoma stand out distinctly from the rest of the liver. Also, the normal arrangement of central veins and portal triads was absent. Sometimes a few portal triads or bile ducts were found at the periphery of the lesion that appeared to be entrapped structures.

Some of the adenomas in this study were atypical in appearance and size. These did not have well-demarcated borders and were composed of variable numbers of eosinophilic, basophilic, clear, or vacuolated hepatocytes and resembled large mixed cell foci involving multiple hepatic lobules. They had well-defined hepatic lobules with central veins and portal triads. These portal triads usually contained bile ductules accompanied by variable oval cell proliferation. Central veins and portal triads within these lesions were more frequent than in typical adenomas. However, because of other histologic features characteristic of benign neoplasia, they were identified as adenoma. These features included the absence of normal lobular architecture, cellular atypia, and increased mitotic activity.

The hepatocellular carcinomas were discrete masses that generally had irregular borders due to localized areas of growth of neoplastic hepatocytes into the surrounding normal parenchyma. They had many of the same features as adenomas but were differentiated from adenomas by the presence of abnormal patterns of growth that resulted in trabeculae that were three or more layers of neoplastic hepatocytes thick. These hepatocytes had cellular atypia and increased mitotic activity. Less commonly they formed glandular structures or areas of solid growth, often combined with hemorrhage, necrosis, and cyst formation. The cytoplasm of neoplastic hepatocytes was eosinophilic, clear, basophilic, vacuolated, or a mixture of these types. As with the adenomas, some carcinomas in this study were atypical in appearance. These had lobular areas with increased numbers of identifiable central veins and portal triads within the carcinomas. Because of other histologic features characteristic of malignant neoplasia including trabeculae that were three or more hepatocyte layers thick or solid areas with marked cellular atypia, they were identified as carcinoma. The carcinomas were typically larger than adenomas. Metastases to the lungs were observed in a few males from all groups including the control group.

Significantly increased incidences of eosinophilic focus or mixed cell focus occurred in all exposed groups of males (Tables 17 and C3). The eosinophilic foci were small to moderately large lesions composed of hepatocytes that were somewhat enlarged and had homogenous or finely granular, eosinophilic cytoplasm. The hepatocytes were arranged in normal lobular patterns in which hepatic cords merged with the surrounding normal hepatocytes. Blood vessels and portal areas were sometimes present within these foci. There was little or no compression of the surrounding normal hepatocytes, although some degree of compression was present in some larger foci. The mixed cell foci were composed of two or more kinds of cell types, with no cell type constituting 80% or more of the focus. Mixed cell foci were often larger than other types of foci and consisted of a mixture of basophilic or eosinophilic cells and clear cells. They had normal lobular hepatocyte patterns that merged into the adjacent parenchyma with variable numbers of blood vessels and portal areas.

Genetic Toxicology

Goldenseal root powder (1,000 to 10,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA100 or TA98 or *Escherichia coli* strain WP2 *uvrA* pKM101 with or without rat liver S9 metabolic activation enzymes (Table E1). No increase in the frequency of micronucleated normochromatic (mature) erythrocytes was observed in peripheral blood samples from male or female B6C3F1 mice exposed to goldenseal root powder in feed (3,121 to 50,000 ppm) for 3 months; no significant exposure-related changes in the percentages of polychromatic (immature) erythrocytes were observed in peripheral blood of these mice, suggesting that no exposure-related bone marrow toxicity occurred (Table E2).

Berberine chloride was also shown to be nongenotoxic. Over a concentration range of 0.33 to 1,000 µg/plate, it was not mutagenic in *Salmonella typhimurium* TA97, TA98, TA100, or TA1535 with or without rat or hamster liver S9 metabolic activation enzymes (Table E3). No increases in the frequency of micronucleated polychromatic erythrocytes were observed in bone marrow samples from male B6C3F1 mice treated with berberine chloride (41.125 to 658 mg/kg) by intraperitoneal injection 3 times at 24 hour intervals; no dose-related change in the percentage of polychromatic erythrocytes in the bone marrow was observed, suggesting that exposure to berberine chloride did not induce bone marrow toxicity (Table E4).

DISCUSSION AND CONCLUSIONS

Goldenseal (*Hydrastis canadensis*) is an herbal medicine widely used in the United States. While goldenseal and berberine (a goldenseal alkaloid) have been used as herbal medicines to treat a variety of ailments including infections and wounds, there are few controlled clinical trials to document clinical effectiveness, and the herb is not licensed as a drug (NCCAM, 2007).

There have been no previous goldenseal 3-month toxicity or 2-year carcinogenicity studies reported in the literature, and, thus, the goldenseal rodent studies reported here are important in evaluating potential sequelae from long-term exposure to the herb. A major finding of these studies was that goldenseal caused treatment-related nonneoplastic lesions and neoplasms in the liver of rats and mice. The exposure concentrations used in the 2-year studies overlapped estimated human exposures. In the 2-year goldenseal study (containing 3.89% berberine), the male rats consumed goldenseal root powder (at 3 months) at approximately 163, 477, or 1,400 mg/kg body weight (Table J1) or 847, 2,480, or 7,280 mg/m^2 body surface area basis for the 3,000, 9,000, or 25,000 ppm groups, respectively, corresponding to approximately 6.3, 18.6, or 54.5 mg/kg of berberine. Male mice consumed approximately 465, 1,403, or 3,997 mg/kg goldenseal root powder (Table J3) or 1,395, 4,209, or 11,991 mg/m^2 body surface area corresponding to 18, 54.5, or 155.5 mg/kg berberine for the 3,000, 9,000, or 25,000 ppm groups, respectively. Female rats and mice consumed approximately the same amount as males based on a mg/kg basis. If a 70 kg man takes 1,000 mg of goldenseal three times a day (NSD, 2007a), he would consume approximately 43 mg goldenseal/kg body weight or 1,586 mg goldenseal/m^2 body surface area basis. (Body surface area exposures were based on the calculations of Freireich *et al*., 1966.)

In the 3-month studies, where goldenseal was administered in the feed at doses up to 50,000 ppm, there were no exposure-related mortality or clinical signs. The liver was identified as a target organ in rats and mice as characterized by centrilobular hypertrophy at exposure concentrations of 6,250 ppm or greater in rats and at 12,500 ppm or greater in mice. The severity of hypertrophy increased with increasing exposure concentration. Hepatocyte hypertrophy consisted of enlarged hepatocytes that can result from increases in smooth endoplasmic reticulum, cytochrome P450 monooxygenase activity, or peroxisome proliferation. It is considered an adaptive response and not evidence of significant cellular damage (Cattley and Popp, 2002). Liver weights were significantly increased in male rats exposed to 6,250 ppm or greater, in all exposed groups of female rats, in male mice exposed to 12,500 ppm or greater, and in female mice exposed to 25,000 or 50,000 ppm. The highest exposure concentration in the 2-year studies was 25,000 ppm, because, at this concentration, the liver findings would not compromise the survival of the animals over a 2-year exposure period.

There was no evidence that goldenseal root powder produced any toxicity to the reproductive system of male or female F344/N rats or B6C3F1 mice in the 3-month study based upon the negative results of the sperm motility and counts and vaginal cytology evaluation and the lack of change in histopathology and reproductive organ weights other than a significant decrease in absolute epididymal weight of 50,000 ppm male mice. Since there was no associated histopathology or effects on sperm counts, the decrease in epididymal weight is not considered biologically relevant.

In the 2-year studies, there were no exposure-related effects on the survival of rats or mice. Mean body weights of exposed groups of male rats and male and female mice and 3,000 and 9,000 ppm female rats were within 10% of those of controls throughout the study; mean body weights of 25,000 ppm female rats had mean body weights reduced up to 20% less that those of controls toward the end of the study.

The primary exposure-related effects in the 2-year rat and mouse studies were neoplastic and nonneoplastic changes in the liver. Exposure-related increases in the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) occurred in male rats, and increased incidences of hepatocellular adenoma occurred in female rats. The incidences of hepatocellu-

lar adenoma and hepatocellular adenoma or hepatocellular carcinoma (combined) in 25,000 ppm males exceeded the current and historical control rates and were considered to be clear evidence of carcinogenic activity. Similarly, the incidences of hepatocellular adenoma in 25,000 ppm females exceeded the current and historical control rates for this neoplasm and were considered to be clear evidence of carcinogenic activity. The finding of exposure-related hepatocellular adenomas in males also supported the finding of clear evidence of carcinogenic activity in females. Evidence from studies reported in the literature show that hepatocellular adenomas are able to progress to malignant tumors (Eguchi *et al*., 1992; Pitot *et al*., 1996; Hussain *et al*., 2002; Takahashi *et al*., 2002; and Suzuki *et al*., 2009).

Nonneoplastic findings in the 2-year rat study included increased incidences of eosinophilic focus in 9,000 and 25,000 ppm males and in all exposed groups of females. The incidences of mixed cell focus, clear cell focus, and basophilic focus in rats had no definite relationship to the exposure concentration. Liver foci are nonneoplastic proliferative lesions with preneoplastic potential (Goldsworthy and Fransson-Steen, 2002, Takahashi *et al*., 2002; Ittrich *et al*., 2003).

In addition to the hepatocytic hypertrophy that was also observed during the 3-month study, hepatocytic degeneration occurred in rats at 2 years. The incidences of hepatocytic degeneration were increased in all exposed groups of males and in 9,000 and 25,000 ppm females; this lesion was characterized by large hepatocytes distended with either finely granular, pale, slightly eosinophilic cytoplasm or numerous small, discrete clear vacuoles as usually seen with fatty changes.

The liver responses during the 2-year mouse study differed from those of the rats in several ways, suggesting mice are less sensitive to the components of goldenseal root powder than rats. Female mice were less affected than males. An exposure-related carcinogenic response occurred in the liver of male mice including increased incidences of hepatocellular adenoma, multiple adenoma, and hepatoblastoma. The incidences of hepatocellular carcinoma were not increased. The incidences of hepatocellular adenoma and hepatoblastoma in 25,000 ppm males exceeded the historical control ranges for feed studies.

Hepatoblastomas are considered to be less-differentiated forms of liver carcinoma. Hepatoblastomas are rare spontaneous neoplasms seen in relatively high numbers only after chemical administration (primarily in mice) and have previously been seen in NTP studies of benzofuran, ethylene thiourea, *o*-nitroanisole, coumarin, oxazepam, methylphenidate hydrochloride, 1-amino-2,4-dibromoanthraquinone, pyridine, and primidone (NTP, 1989, 1992, 1993a,b,c, 1995, 1996, 2000a,b). They often arise from hepatocellular neoplasms and when this occurs, only the hepatoblastoma is diagnosed. Hepatoblastomas in humans account for approximately 70% of childhood liver cancers (Ding *et al*., 1994).

Hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma represent a biological and morphological continuum (Takahashi *et al.,* 2002); therefore, it is appropriate to combine the incidences of hepatoblastoma with those of hepatocellular adenoma and carcinoma when interpreting the carcinogenic potential of a chemical.

Because the control male mice had a relatively high spontaneous incidence of hepatocellular adenoma when compared to historical controls, the increased liver carcinogenic response in exposed groups of males was considered some evidence of carcinogenic activity. This opinion was based upon the increased incidence of hepatoblastoma in the 25,000 ppm group and the increased incidences of multiple hepatocellular adenoma in all exposed groups. In addition, there was a decreased time for the first observation of hepatocellular adenomas (including multiple) and the incidence of hepatoblastoma in 25,000 ppm males exceeded the rates in the current controls and the historical controls (feed studies).

In mice, the incidences of eosinophilic focus were increased in 3,000 and 9,000 ppm males and significantly increased in 25,000 ppm males. The hepatocytic degeneration and hypertrophy seen in rats were not observed in mice.

In contrast to the findings in male mice, the incidences of liver neoplasms in exposed female mice were not increased. This sex difference in the mouse liver tumor response is also seen in humans, where liver neoplastic responses occur more frequently in males than in females (Howe *et al*., 2006; Wands, 2007). Studies suggest that this sex difference may be related to estrogenic responses. Administration of estrogen reduced the rate of diethylnitrosamine hepatocarcinogenesis in male mice (Nakatani *et al*., 2001). Naugler *et al*. (2007) proposed that an estrogenic liver carcinogenic protective effect

may be related to the level of the proinflammatory cytokine interleukin-6. In interleukin-6 knockout male mice, the administration of diethylnitrosamine results in a lower incidence of hepatocellular carcinoma and much higher odds of survival than in diethylnitrosamine treated "wild" mice (Naugler *et al*., 2007; Wands, 2007). The authors suggest that estrogen-mediated inhibition of interleukin-6 production reduces the liver cancer risk in females (Naugler *et al*., 2007).

Protoberberine alkaloids, including berberine and some of its metabolites (Zuo *et al*., 2006) disrupt DNA repair processes (Mazzini *et al*., 2003) by interfering with topoisomerase I activity (Li *et al*., 2000; Kettmann *et al*., 2004b); berberine may also inhibit topoisomerase II (Krishnan and Bastow, 2000). The berberine metabolite, berberrubine is reported to be a topoisomerase II inhibitor (Kobayashi *et al*., 1995; Makhey *et al*., 1994) and the metabolite demethyleneberberine is a topoisomerase I inhibitor (Makhey *et al*., 1994). Topoisomerase inhibition, in addition to causing cell death, may also induce chromosomal aberrations that can, in turn, result in malignancies (Malik *et al*., 2006). The essential components for DNA repair processes are conserved among eukaryotes (Malik *et al*., 2006), and a chemical that inhibits DNA repair in rodents may also inhibit DNA repair in other mammalian species. The liver of the rat is one of the tissues with the highest rates of DNA repair (Gospodinov *et al*., 2003), and thus, disruption of DNA repair in this tissue might make it more susceptible to exogenous oxidative stress than other tissues. In B6C3F1 mice, the liver is predisposed to neoplasm formation, and therefore, disruption of DNA repair processes may enhance carcinogenic events in this organ.

IARC (2000) reported that the topoisomerase inhibitors, etoposide and teniposide, are group 2A carcinogens (probably carcinogenic to humans) based on human data showing that use of these drugs can lead to secondary cancers; however, there have been no adequate animal carcinogenicity studies for these topoisomerase inhibitors. The current studies of goldenseal root powder, which contains the topoisomerase inhibitor berberine, suggest that additional studies of these topoisomerase inhibitors may be warranted.

Studies in the literature have reported that goldenseal or its alkaloid, berberine, may improve cardiac function. Berberine has been reported to decrease toxicity in rat isolated mesenteric arteries (Lee and Chang, 1996; Ko *et al*., 2000). A goldenseal extract was reported to block calcium channels and adrenoceptors in a rat aorta preparation (Lee and Chang, 1996) and ATP-sensitive potassium channels in cardiac myocytes (Wang *et al*., 1996). When Sprague-Dawley rats with cardiac hypertrophy induced by suprarenal abdominal aorta constriction received berberine for 8 weeks (oral administration; 10 mg/kg per day), the treatment inhibited the development of cardiohypertrophy (as determine by heart weight) and decreased noradrenaline plasma levels and adrenaline plasma and left ventricular levels (Hong *et al*., 2003). The current study also suggests that goldenseal may reduce the background level of cardiomyopathy in the F344/N rats.

Studies in humans suggest that goldenseal alkaloids may protect against cardiac disease. In one study in China, 156 patients with chronic congestive heart disease were randomized into two groups. Both groups received conventional therapy of angiotensin-converting enzyme inhibitors, digoxin, diuretics, and nitrates. One group received, in addition, berberine at 1.2 to 2.0 g/day (for a 70 kg person, up to approximately 30 mg/kg per day). After treatment with berberine for 8 weeks, there was a significantly greater increase in left ventricular ejection fraction, exercise capacity, and a decrease in ventricular premature complexes than in the control group receiving the conventional therapy alone (Zeng *et al*., 2003). In another study, when patients received berberine in addition to conventional heart therapy there was a reduction in ventricular premature heart beats with berberine plasma levels reaching 0.11 mg/L or greater (Zeng and Zeng, 1999).

In the heart, adrenaline binds beta-2 adrenergic receptors at the catechol hydroxyl groups, the amine group, and the alkyl hydroxyl group thus triggering the cAMP cascade (Goddard and Abrol, 2007). One hypothesis for the goldenseal effects in reducing cardiac damage is that a berberine metabolite, demethyleneberberine, which contains a catechol as well as an amine center, is structurally similar to adrenergic receptor antagonists, and may mimic these antagonists in blocking the effects of the adrenaline/beta-2 adrenergic receptor activities.

Significant decreases in the incidences of mammary gland hyperplasia and mammary gland fibroadenoma occurred in female rats. Because the mean body weights of 25,000 ppm female rats were reduced by up to 20% that of the controls toward the end of the study, the relationship between body weight reduction and the reduction in mammary gland neoplasms was analyzed by the

model of Haseman *et al* (1997). This model predicts mammary gland neoplasms in 18.8, 17.5, 15.4, and 13.0 animals for the four exposure groups, respectively, based on body weight. It is difficult to draw a definitive conclusion about body weight effects on the development of mammary gland neoplasms in the current study, because the mammary gland neoplasm rates in all groups except the 9,000 ppm group are higher than would be predicted by the model. However, the model predicted a 31% reduction in mammary gland neoplasms in the 25,000 ppm group based on body weight effects alone (13 in the 25,000 ppm group versus 18.8 in the control group), and the actual reduction was 39% (17 in the 25,000 ppm group versus 30 in the control group), suggesting that body weight may be a factor in the reduction of the incidences of this neoplasm.

Because goldenseal is a mixture of several alkaloids, it is not possible to clearly attribute the effects noted in this study to any one of the constituent alkaloids.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity** of goldenseal root powder in male F344/N rats based on the increased incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined). There was *clear evidence of carcinogenic activity* of goldenseal root powder in female F344/N rats based on the increased incidence of hepatocellular adenoma. There was *some evidence of carcinogenic activity* of goldenseal root powder in male B6C3F1 mice based on the increased incidences of hepatoblastoma and multiple hepatocellular adenoma. There was *no evidence of carcinogenic activity* of goldenseal root powder in female B6C3F1 mice exposed to 3,000, 9,000, or 25,000 ppm goldenseal root powder in feed for 2 years.

Administration of goldenseal root powder resulted in increased incidences of nonneoplastic lesions in the liver of male and female rats and male mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF GOLDENSEAL ROOT POWDER

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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	19	11	24
Natural deaths	5	2	9	1
Survivors				
Died last week of study				1
Terminal sacrifice	33	29	30	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(49)	(50)	(48)	(50)
Intestine large, colon	(50)	(48)	(50)	(50)
Intestine small, duodenum	(49)	(49)	(49)	(49)
Carcinoma				1 (2%)
Intestine small, ileum	(47)	(49)	(45)	(48)
Intestine small, jejunum	(48)	(48)	(46)	(48)
Adenoma				1 (2%)
Carcinoma			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	1 (2%)	1 (2%)	2 (4%)	8 (16%)
Hepatocellular adenoma, multiple				2 (4%)
Hepatocellular carcinoma				1 (2%)
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)			
Mesentery	(9)	(9)	(11)	(7)
Lipoma	1 (11%)			
Oral mucosa	(1)	(0)	(0)	(0)
Squamous cell carcinoma	1 (100%)			
Pancreas	(50)	(50)	(49)	(50)
Mixed tumor malignant	1 (2%)			
Acinus, adenoma				1 (2%)
Salivary glands	(50)	(49)	(50)	(50)
Schwannoma malignant				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(49)
Tongue	(2)	(1)	(0)	(0)
Squamous cell papilloma	1 (50%)	1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Schwannoma malignant			2 (4%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Endocrine System (continued)				
Adrenal medulla	(49)	(49)	(50)	(48)
Pheochromocytoma benign	4 (8%)	7 (14%)	7 (14%)	4 (8%)
Pheochromocytoma benign, multiple	1 (2%)			1 (2%)
Pheochromocytoma malignant	1 (2%)	5 (10%)	2 (4%)	
Pheochromocytoma malignant, multiple		1 (2%)		
Islets, pancreatic	(50)	(50)	(49)	(50)
Adenoma	6 (12%)	3 (6%)	4 (8%)	2 (4%)
Adenoma, multiple	1 (2%)			
Carcinoma			1 (2%)	
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, adenoma	8 (16%)	18 (36%)	16 (33%)	13 (26%)
Pars distalis, carcinoma		1 (2%)		
Pars intermedia, adenoma			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	7 (14%)	4 (8%)	6 (12%)	3 (6%)
C-cell, adenoma, multiple		1 (2%)		
C-cell, carcinoma	1 (2%)	2 (4%)		1 (2%)
C-cell, carcinoma, multiple		1 (2%)		
Follicular cell, adenoma				1 (2%)
General Body System				
Tissue NOS	(0)	(1)	(1)	(0)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma		3 (6%)	2 (4%)	2 (4%)
Carcinoma	2 (4%)	2 (4%)	1 (2%)	4 (8%)
Prostate	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	31 (62%)	31 (62%)	38 (76%)	33 (66%)
Interstitial cell, adenoma	11 (22%)	12 (24%)	6 (12%)	9 (18%)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Lymph node	(11)	(11)	(14)	(19)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (5%)
Deep cervical, carcinoma, metastatic, thyroid gland		2 (18%)		
Deep cervical, rhabdomyosarcoma, metastatic, harderian gland		1 (9%)		
Inguinal, alveolar/bronchiolar carcinoma, metastatic, lung				1 (5%)
Mediastinal, carcinoma, metastatic, thyroid gland		1 (9%)		
Mediastinal, rhabdomyosarcoma, metastatic, harderian gland		1 (9%)		
Lymph node, mesenteric	(49)	(49)	(50)	(50)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Hematopoietic System (continued)				
Spleen	(49)	(50)	(49)	(50)
Fibrous histiocytoma	1 (2%)			
Thymus	(48)	(49)	(49)	(47)
Thymoma benign	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibroadenoma	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Fibroadenoma, multiple			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma			1 (2%)	
Basal cell carcinoma		1 (2%)		2 (4%)
Keratoacanthoma	2 (4%)	4 (8%)	5 (10%)	2 (4%)
Sebaceous gland, adenoma				2 (4%)
Subcutaneous tissue, fibroma	4 (8%)	4 (8%)	2 (4%)	5 (10%)
Subcutaneous tissue, fibroma, multiple	1 (2%)			
Subcutaneous tissue, fibrosarcoma			2 (4%)	
Subcutaneous tissue, lipoma	1 (2%)		2 (4%)	
Subcutaneous tissue, myxosarcoma		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chordoma	1 (2%)			1 (2%)
Osteosarcoma	2 (4%)		1 (2%)	
Skeletal muscle	(3)	(1)	(1)	(3)
Rhabdomyosarcoma	1 (33%)			
Nervous System				
Brain	(49)	(50)	(50)	(50)
Astrocytoma malignant				1 (2%)
Ependymoma malignant	1 (2%)			
Granular cell tumor malignant		1 (2%)		
Oligodendroglioma malignant			1 (2%)	
Peripheral nerve	(4)	(3)	(4)	(3)
Schwannoma malignant				1 (33%)
Spinal cord	(4)	(3)	(4)	(2)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		2 (4%)		2 (4%)
Alveolar/bronchiolar carcinoma		1 (2%)		1 (2%)
Carcinoma	1 (2%)			
Carcinoma, metastatic, thyroid gland		1 (2%)		
Chordoma, metastatic, bone				1 (2%)
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		
Nose	(50)	(50)	(50)	(50)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Rhabdomyosarcoma, metastatic, harderian gland		1 (2%)		
Harderian gland	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Rhabdomyosarcoma		1 (2%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma			1 (2%)	
Transitional epithelium, carcinoma	1 (2%)			
Urethra	(0)	(1)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	2 (4%)	1 (2%)
Leukemia mononuclear	21 (42%)	16 (32%)	24 (48%)	28 (56%)
Mesothelioma malignant		3 (6%)	2 (4%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	50	50	49
Total primary neoplasms	118	130	140	138
Total animals with benign neoplasms	47	48	48	47
Total benign neoplasms	83	93	101	95
Total animals with malignant neoplasms	29	29	31	37
Total malignant neoplasms	35	37	39	43
Total animals with metastatic neoplasms	1	3		3
Total metastatic neoplasms	1	8		5
Total animals with malignant neoplasms of uncertain primary site				1

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

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	5/49 (10%)	7/49 (14%)	7/50 (14%)	5/48 (10%)
Adjusted rate[b]	11.9%	17.1%	15.9%	12.7%
Terminal rate[c]	4/33 (12%)	6/28 (21%)	3/30 (10%)	3/25 (12%)
First incidence (days)	600	645	659	653
Poly-3 test[d]	P=0.507N	P=0.355	P=0.409	P=0.586
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	1/49 (2%)	6/49 (12%)	2/50 (4%)	0/48 (0%)
Adjusted rate	2.4%	14.3%	4.6%	0.0%
Terminal rate	1/33 (3%)	4/28 (14%)	1/30 (3%)	0/25 (0%)
First incidence (days)	729 (T)	506	701	—[e]
Poly-3 test	P=0.091N	P=0.055	P=0.514	P=0.514N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	6/49 (12%)	13/49 (27%)	9/50 (18%)	5/48 (10%)
Adjusted rate	14.2%	30.8%	20.4%	12.7%
Terminal rate	5/33 (15%)	10/28 (36%)	4/30 (13%)	3/25 (12%)
First incidence (days)	600	506	659	653
Poly-3 test	P=0.190N	P=0.056	P=0.319	P=0.550N
Liver: Hepatocellular Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	10/50 (20%)
Adjusted rate	2.4%	2.4%	4.6%	24.2%
Terminal rate	1/33 (3%)	0/29 (0%)	1/30 (3%)	5/25 (20%)
First incidence (days)	729 (T)	684	663	612
Poly-3 test	P<0.001	P=0.758	P=0.511	P=0.003
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	11/50 (22%)
Adjusted rate	2.4%	2.4%	4.6%	26.5%
Terminal rate	1/33 (3%)	0/29 (0%)	1/30 (3%)	5/25 (20%)
First incidence (days)	729 (T)	684	663	612
Poly-3 test	P<0.001	P=0.758	P=0.511	P<0.001
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.4%	7.2%	0.0%	7.5%
Terminal rate	1/33 (3%)	2/29 (7%)	0/30 (0%)	3/25 (12%)
First incidence (days)	729 (T)	723	—	729 (T)
Poly-3 test	P=0.309	P=0.299	P=0.495N	P=0.286
Mammary Gland: Fibroadenoma				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	2.4%	2.4%	9.2%	2.5%
Terminal rate	1/33 (3%)	1/29 (3%)	3/30 (10%)	1/25 (4%)
First incidence (days)	729 (T)	729 (T)	701	729 (T)
Poly-3 test	P=0.608	P=0.757	P=0.187	P=0.749
Pancreatic Islets: Adenoma				
Overall rate	7/50 (14%)	3/50 (6%)	4/49 (8%)	2/50 (4%)
Adjusted rate	16.1%	7.1%	9.3%	5.0%
Terminal rate	4/33 (12%)	1/29 (3%)	1/30 (3%)	1/25 (4%)
First incidence (days)	571	663	696	684
Poly-3 test	P=0.126N	P=0.169N	P=0.263N	P=0.096N

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	3/50 (6%)	5/49 (10%)	2/50 (4%)
Adjusted rate	16.1%	7.1%	11.6%	5.0%
Terminal rate	4/33 (12%)	1/29 (3%)	2/30 (7%)	1/25 (4%)
First incidence (days)	571	663	696	684
Poly-3 test	P=0.134N	P=0.169N	P=0.383N	P=0.096N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	8/50 (16%)	18/50 (36%)	16/49 (33%)	13/50 (26%)
Adjusted rate	18.1%	40.9%	36.3%	31.1%
Terminal rate	4/33 (12%)	10/29 (35%)	11/30 (37%)	8/25 (32%)
First incidence (days)	407	437	563	523
Poly-3 test	P=0.373	P=0.014	P=0.043	P=0.123
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	8/50 (16%)	19/50 (38%)	16/49 (33%)	13/50 (26%)
Adjusted rate	18.1%	43.2%	36.3%	31.1%
Terminal rate	4/33 (12%)	11/29 (38%)	11/30 (37%)	8/25 (32%)
First incidence (days)	407	437	563	523
Poly-3 test	P=0.413	P=0.008	P=0.043	P=0.123
Preputial Gland: Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	0.0%	7.1%	4.6%	5.0%
Terminal rate	0/33 (0%)	2/29 (7%)	1/30 (3%)	2/25 (8%)
First incidence (days)	—	565	701	729 (T)
Poly-3 test	P=0.405	P=0.117	P=0.243	P=0.225
Preputial Gland: Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	1/50 (2%)	4/50 (8%)
Adjusted rate	4.7%	4.8%	2.3%	9.7%
Terminal rate	2/33 (6%)	1/29 (3%)	1/30 (3%)	1/25 (4%)
First incidence (days)	729 (T)	642	729 (T)	523
Poly-3 test	P=0.194	P=0.691	P=0.491N	P=0.326
Preputial Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	3/50 (6%)	6/50 (12%)
Adjusted rate	4.7%	11.8%	6.9%	14.5%
Terminal rate	2/33 (6%)	3/29 (10%)	2/30 (7%)	3/25 (12%)
First incidence (days)	729 (T)	565	701	523
Poly-3 test	P=0.154	P=0.216	P=0.513	P=0.124
Prostate Gland: Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.4%	2.4%	6.9%	5.0%
Terminal rate	1/33 (3%)	1/29 (3%)	3/30 (10%)	1/25 (4%)
First incidence (days)	729 (T)	729 (T)	729 (T)	703
Poly-3 test	P=0.363	P=0.757	P=0.314	P=0.482
Skin: Keratoacanthoma				
Overall rate	2/50 (4%)	4/50 (8%)	5/50 (10%)	2/50 (4%)
Adjusted rate	4.7%	9.6%	11.5%	5.0%
Terminal rate	1/33 (3%)	4/29 (14%)	5/30 (17%)	1/25 (4%)
First incidence (days)	456	729 (T)	729 (T)	688
Poly-3 test	P=0.478N	P=0.320	P=0.219	P=0.670

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Skin: Keratoacanthoma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	6/50 (12%)	4/50 (8%)
Adjusted rate	4.7%	12.0%	13.7%	9.9%
Terminal rate	1/33 (3%)	5/29 (17%)	5/30 (17%)	2/25 (8%)
First incidence (days)	456	729 (T)	659	635
Poly-3 test	P=0.419	P=0.201	P=0.136	P=0.310
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	5/50 (10%)	4/50 (8%)	2/50 (4%)	5/50 (10%)
Adjusted rate	11.8%	9.5%	4.6%	12.4%
Terminal rate	4/33 (12%)	2/29 (7%)	2/30 (7%)	4/25 (16%)
First incidence (days)	656	563	729 (T)	627
Poly-3 test	P=0.485	P=0.507N	P=0.207N	P=0.599
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Myxosarcoma				
Overall rate	5/50 (10%)	5/50 (10%)	4/50 (8%)	5/50 (10%)
Adjusted rate	11.8%	11.8%	9.2%	12.4%
Terminal rate	4/33 (12%)	2/29 (7%)	3/30 (10%)	4/25 (16%)
First incidence (days)	656	563	696	627
Poly-3 test	P=0.543	P=0.626	P=0.486N	P=0.599
Testes: Adenoma				
Overall rate	42/50 (84%)	43/50 (86%)	44/50 (88%)	42/50 (84%)
Adjusted rate	90.7%	91.8%	91.5%	91.7%
Terminal rate	32/33 (97%)	28/29 (97%)	28/30 (93%)	25/25 (100%)
First incidence (days)	456	506	431	478
Poly-3 test	P=0.549	P=0.583	P=0.599	P=0.593
Thyroid Gland (C-Cell): Adenoma				
Overall rate	7/50 (14%)	5/50 (10%)	6/50 (12%)	3/50 (6%)
Adjusted rate	16.5%	11.8%	13.8%	7.3%
Terminal rate	5/33 (15%)	2/29 (7%)	4/30 (13%)	1/25 (4%)
First incidence (days)	711	632	688	547
Poly-3 test	P=0.169N	P=0.378N	P=0.479N	P=0.168N
Thyroid Gland (C-Cell): Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.4%	7.1%	0.0%	2.5%
Terminal rate	1/33 (3%)	1/29 (3%)	0/30 (0%)	1/25 (4%)
First incidence (days)	729 (T)	632	—	729 (T)
Poly-3 test	P=0.433N	P=0.304	P=0.495N	P=0.749
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	8/50 (16%)	7/50 (14%)	6/50 (12%)	4/50 (8%)
Adjusted rate	18.9%	16.4%	13.8%	9.7%
Terminal rate	6/33 (18%)	3/29 (10%)	4/30 (13%)	2/25 (8%)
First incidence (days)	711	632	688	547
Poly-3 test	P=0.156N	P=0.494N	P=0.364N	P=0.189N
All Organs: Mononuclear Cell Leukemia				
Overall rate	21/50 (42%)	16/50 (32%)	24/50 (48%)	28/50 (56%)
Adjusted rate	45.5%	35.4%	50.3%	61.4%
Terminal rate	11/33 (33%)	5/29 (17%)	11/30 (37%)	12/25 (48%)
First incidence (days)	456	355	316	478
Poly-3 test	P=0.018	P=0.220N	P=0.397	P=0.089

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
All Organs: Malignant Mesothelioma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	0.0%	7.2%	4.6%	0.0%
Terminal rate	0/33 (0%)	3/29 (10%)	1/30 (3%)	0/25 (0%)
First incidence (days)	—	729 (T)	709	—
Poly-3 test	P=0.319N	P=0.115	P=0.243	—[f]
All Organs: Benign Neoplasms				
Overall rate	47/50 (94%)	48/50 (96%)	48/50 (96%)	47/50 (94%)
Adjusted rate	97.4%	99.1%	97.8%	98.7%
Terminal rate	33/33 (100%)	29/29 (100%)	29/30 (97%)	25/25 (100%)
First incidence (days)	407	437	431	450
Poly-3 test	P=0.589	P=0.596	P=0.740	P=0.662
All Organs: Malignant Neoplasms				
Overall rate	29/50 (58%)	29/50 (58%)	31/50 (62%)	37/50 (74%)
Adjusted rate	59.1%	60.6%	62.9%	76.6%
Terminal rate	14/33 (42%)	12/29 (41%)	13/30 (43%)	15/25 (60%)
First incidence (days)	422	355	316	208
Poly-3 test	P=0.029	P=0.524	P=0.429	P=0.049
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	50/50 (100%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%	99.5%
Terminal rate	33/33 (100%)	29/29 (100%)	30/30 (100%)	25/25 (100%)
First incidence (days)	407	355	316	208
Poly-3 test	P=0.967N	—	—	P=0.999N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreatic islets, pituitary gland, preputial gland, prostate 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 control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of the statistic cannot be computed

TABLE A3a
Historical Incidence of Hepatocellular Neoplasms in Control Male F344/N Rats[a]

Study	Incidence in Controls	
	Adenoma	Adenoma or Carcinoma
Historical Incidence: Feed Studies		
Benzophenone	2/50	2/50
Chromium picolinate monohydrate	1/50	1/50
Cresols	0/50	0/50
Goldenseal root powder	1/50	1/50
4-Methylimidazole	0/50	0/50
Milk thistle extract	3/50	3/50
Total (%)	7/300 (2.3%)	7/300 (2.3%)
Mean ± standard deviation	2.3% ± 2.3%	2.3% ± 2.3%
Range	0%-6%	0%-6%
Overall Historical Incidence: All Routes		
Total (%)	17/1,399 (1.2%)	22/1,399 (1.6%)
Mean ± standard deviation	1.2% ± 1.7%	1.6% ± 1.7%
Range	0%-6%	0%-6%

[a] Data as of November 17, 2008

TABLE A3b
Historical Incidence of Pituitary Gland (Pars Distalis) Adenoma in Control Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Feed Studies	
Benzophenone	10/50
Chromium picolinate monohydrate	10/50
Cresols	20/50
Goldenseal root powder	8/50
4-Methylimidazole	16/49
Milk thistle extract	13/50
Total (%)	77/299 (25.8%)
Mean ± standard deviation	25.8% ± 9.1%
Range	16%-40%
Overall Historical Incidence: All Routes	
Total (%)	635/1,394 (45.6%)
Mean ± standard deviation	45.6% ± 20.7%
Range	12%-74%

[a] Data as of November 17, 2008

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	19	11	24
Natural deaths	5	2	9	1
Survivors				
Died last week of study				1
Terminal sacrifice	33	29	30	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(49)	(50)	(48)	(50)
Edema	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Intestine large, colon	(50)	(48)	(50)	(50)
Intestine small, duodenum	(49)	(49)	(49)	(49)
Intestine small, ileum	(47)	(49)	(45)	(48)
Inflammation, chronic				1 (2%)
Intestine small, jejunum	(48)	(48)	(46)	(48)
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	2 (4%)	
Atrophy, focal	1 (2%)			
Basophilic focus	20 (40%)	33 (66%)	22 (44%)	13 (26%)
Clear cell focus	19 (38%)	17 (34%)	3 (6%)	2 (4%)
Degeneration, cystic	6 (12%)	4 (8%)	4 (8%)	7 (14%)
Eosinophilic focus	4 (8%)	5 (10%)	25 (50%)	28 (56%)
Hematopoietic cell proliferation		1 (2%)		
Hemorrhage	1 (2%)	6 (12%)		
Hepatodiaphragmatic nodule	3 (6%)	13 (26%)	6 (12%)	2 (4%)
Infarct				1 (2%)
Infiltration cellular, mixed cell	4 (8%)	6 (12%)	6 (12%)	6 (12%)
Mixed cell focus	9 (18%)	21 (42%)	13 (26%)	7 (14%)
Necrosis, focal	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Bile duct, hyperplasia	43 (86%)	38 (76%)	45 (90%)	39 (78%)
Centrilobular, necrosis		2 (4%)		1 (2%)
Hepatocyte, degeneration		22 (44%)	30 (60%)	19 (38%)
Hepatocyte, hyperplasia, focal	1 (2%)			
Hepatocyte, hypertrophy		19 (38%)	31 (62%)	27 (54%)
Hepatocyte, vacuolization cytoplasmic	2 (4%)	4 (8%)	2 (4%)	1 (2%)
Kupffer cell, pigmentation	2 (4%)		2 (4%)	3 (6%)
Mesentery	(9)	(9)	(11)	(7)
Accessory spleen	3 (33%)		1 (9%)	1 (14%)
Angiectasis			1 (9%)	
Hemorrhage			2 (18%)	
Inflammation, chronic	1 (11%)			
Fat, necrosis	4 (44%)	5 (56%)	7 (64%)	3 (43%)
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(50)	(50)	(49)	(50)
Atrophy	19 (38%)	19 (38%)	25 (51%)	18 (36%)
Cyst	10 (20%)	12 (24%)	15 (31%)	14 (28%)
Necrosis			1 (2%)	
Acinus, cytoplasmic alteration	1 (2%)	3 (6%)		1 (2%)
Acinus, hyperplasia, focal	6 (12%)		5 (10%)	1 (2%)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Alimentary System (continued)				
Salivary glands	(50)	(49)	(50)	(50)
Atrophy	4 (8%)	2 (4%)	4 (8%)	6 (12%)
Infiltration cellular, polymorphonuclear			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	2 (4%)	5 (10%)	2 (4%)	1 (2%)
Erosion		1 (2%)		
Hyperplasia, squamous			1 (2%)	
Inflammation, chronic active		1 (2%)	1 (2%)	
Perforation			1 (2%)	
Ulcer	3 (6%)	8 (16%)	2 (4%)	1 (2%)
Epithelium, hyperplasia	5 (10%)	7 (14%)	3 (6%)	3 (6%)
Stomach, glandular	(50)	(50)	(50)	(49)
Erosion	3 (6%)	2 (4%)	3 (6%)	3 (6%)
Ulcer		3 (6%)	2 (4%)	
Epithelium, hyperplasia	3 (6%)		1 (2%)	
Tongue	(2)	(1)	(0)	(0)
Epithelium, hyperplasia	1 (50%)			
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	47 (94%)	38 (76%)	39 (78%)	36 (72%)
Thrombosis	1 (2%)		1 (2%)	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	17 (34%)	7 (14%)	14 (28%)	19 (38%)
Degeneration, fatty		1 (2%)		
Hyperplasia	10 (20%)	2 (4%)	5 (10%)	7 (14%)
Hyperplasia, focal	3 (6%)	2 (4%)	4 (8%)	2 (4%)
Hypertrophy, focal	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Necrosis	1 (2%)		1 (2%)	1 (2%)
Vacuolization cytoplasmic, focal	2 (4%)	6 (12%)	3 (6%)	4 (8%)
Vacuolization cytoplasmic, diffuse		1 (2%)	1 (2%)	1 (2%)
Adrenal medulla	(49)	(49)	(50)	(48)
Hyperplasia	5 (10%)	10 (20%)	14 (28%)	9 (19%)
Islets, pancreatic	(50)	(50)	(49)	(50)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, angiectasis	2 (4%)	4 (8%)	2 (4%)	5 (10%)
Pars distalis, cyst	7 (14%)		6 (12%)	4 (8%)
Pars distalis, hemorrhage, chronic	1 (2%)			
Pars distalis, hyperplasia, focal	14 (28%)	6 (12%)	8 (16%)	10 (20%)
Pars intermedia, cyst	3 (6%)	2 (4%)	2 (4%)	5 (10%)
Thyroid gland	(50)	(50)	(50)	(50)
Ultimobranchial cyst			1 (2%)	1 (2%)
C-cell, hyperplasia	8 (16%)	4 (8%)	4 (8%)	6 (12%)
Follicle, cyst	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Follicular cell, hyperplasia				1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
General Body System				
Tissue NOS	(0)	(1)	(1)	(0)
Hemorrhage		1 (100%)		
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)			2 (4%)
Inflammation, acute		1 (2%)	1 (2%)	
Inflammation, chronic	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Preputial gland	(50)	(50)	(50)	(50)
Cyst	2 (4%)	1 (2%)	3 (6%)	
Hyperplasia	2 (4%)		2 (4%)	1 (2%)
Hyperplasia, focal	1 (2%)	1 (2%)		
Inflammation, chronic	19 (38%)	10 (20%)	14 (28%)	20 (40%)
Prostate	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Inflammation, acute				1 (2%)
Inflammation, chronic	12 (24%)	15 (30%)	14 (28%)	10 (20%)
Epithelium, hyperplasia	4 (8%)	4 (8%)	5 (10%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation, chronic				1 (2%)
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, atrophy	5 (10%)	8 (16%)	3 (6%)	8 (16%)
Interstitial cell, hyperplasia	2 (4%)	6 (12%)	4 (8%)	3 (6%)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Hyperplasia	4 (8%)	4 (8%)	4 (8%)	8 (16%)
Infiltration cellular, histiocyte			2 (4%)	
Myelofibrosis	2 (4%)	2 (4%)		3 (6%)
Lymph node	(11)	(11)	(14)	(19)
Deep cervical, hyperplasia	1 (9%)			
Mediastinal, ectasia	1 (9%)		2 (14%)	1 (5%)
Mediastinal, hemorrhage	1 (9%)	2 (18%)	1 (7%)	1 (5%)
Mediastinal, hyperplasia, lymphoid	3 (27%)	1 (9%)	2 (14%)	3 (16%)
Mediastinal, pigmentation		1 (9%)		1 (5%)
Pancreatic, ectasia		1 (9%)	1 (7%)	1 (5%)
Pancreatic, hemorrhage	1 (9%)		1 (7%)	1 (5%)
Pancreatic, hyperplasia, lymphoid		1 (9%)		1 (5%)
Lymph node, mesenteric	(49)	(49)	(50)	(50)
Atrophy				1 (2%)
Ectasia	2 (4%)	1 (2%)	4 (8%)	2 (4%)
Hemorrhage		2 (4%)	2 (4%)	1 (2%)
Hyperplasia, lymphoid	4 (8%)	11 (22%)	9 (18%)	8 (16%)
Infiltration cellular, mixed cell		1 (2%)		
Spleen	(49)	(50)	(49)	(50)
Accessory spleen		1 (2%)		
Fibrosis	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Hematopoietic cell proliferation	2 (4%)	8 (16%)	4 (8%)	11 (22%)
Hemorrhage			1 (2%)	2 (4%)
Hyperplasia, lymphoid			2 (4%)	1 (2%)
Infiltration cellular, mixed cell		3 (6%)	1 (2%)	
Metaplasia, lipocyte		1 (2%)		
Necrosis			3 (6%)	1 (2%)

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Hematopoietic System (continued)				
Thymus	(48)	(49)	(49)	(47)
Atrophy				2 (4%)
Cyst		2 (4%)		
Fibrosis		1 (2%)		
Hyperplasia, lymphoid	1 (2%)			
Epithelial cell, hyperplasia		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	7 (14%)	13 (26%)	6 (12%)	8 (16%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Edema		2 (4%)		
Hyperkeratosis	4 (8%)	2 (4%)	4 (8%)	1 (2%)
Inflammation, chronic	2 (4%)		2 (4%)	
Ulcer	1 (2%)		1 (2%)	1 (2%)
Epidermis, hyperplasia	5 (10%)		4 (8%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fracture				1 (2%)
Skeletal muscle	(3)	(1)	(1)	(3)
Atrophy	1 (33%)			1 (33%)
Nervous System				
Brain	(49)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Compression	2 (4%)	10 (20%)	3 (6%)	4 (8%)
Gliosis				1 (2%)
Hemorrhage	2 (4%)	4 (8%)	2 (4%)	1 (2%)
Hydrocephalus			1 (2%)	
Metaplasia, lipocyte				1 (2%)
Necrosis	1 (2%)	4 (8%)	1 (2%)	
Peripheral nerve	(4)	(3)	(4)	(3)
Spinal cord	(4)	(3)	(4)	(2)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Edema			1 (2%)	
Emphysema	1 (2%)			
Foreign body		1 (2%)	1 (2%)	
Hemorrhage	1 (2%)	4 (8%)	4 (8%)	4 (8%)
Infiltration cellular, histiocyte	34 (68%)	24 (48%)	25 (50%)	24 (48%)
Inflammation, suppurative		1 (2%)		
Inflammation, chronic	16 (32%)	10 (20%)	17 (34%)	3 (6%)
Metaplasia, osseous	5 (10%)	3 (6%)		1 (2%)
Alveolar epithelium, hyperplasia	8 (16%)	3 (6%)	3 (6%)	8 (16%)
Nose	(50)	(50)	(50)	(50)
Foreign body	6 (12%)	5 (10%)	5 (10%)	6 (12%)
Inflammation, chronic	12 (24%)	4 (8%)	3 (6%)	3 (6%)
Respiratory epithelium, hyperplasia	5 (10%)	3 (6%)	3 (6%)	3 (6%)
Respiratory epithelium, metaplasia, squamous	2 (4%)			

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	2 (4%)			1 (2%)
Inflammation, chronic		1 (2%)		
Retina, degeneration	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)		1 (2%)	1 (2%)
Inflammation, chronic		1 (2%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst		2 (4%)		1 (2%)
Hydronephrosis				1 (2%)
Infarct			3 (6%)	2 (4%)
Inflammation, suppurative	1 (2%)			
Nephropathy	46 (92%)	49 (98%)	46 (92%)	49 (98%)
Renal tubule, accumulation, hyaline droplet	2 (4%)			1 (2%)
Renal tubule, mineralization				1 (2%)
Renal tubule, necrosis	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Renal tubule, pigmentation	3 (6%)	1 (2%)	4 (8%)	4 (8%)
Transitional epithelium, hyperplasia		2 (4%)		
Urethra	(0)	(1)	(0)	(0)
Angiectasis		1 (100%)		
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		1 (2%)
Inflammation, acute				1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF GOLDENSEAL ROOT POWDER

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TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	14	9	5	12
Natural deaths	6	5	3	2
Survivors				
Terminal sacrifice	30	36	42	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(48)	(50)	(50)	(49)
Intestine small, jejunum	(46)	(47)	(48)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma			1 (2%)	8 (16%)
Mesentery	(9)	(7)	(4)	(2)
Oral mucosa	(0)	(0)	(1)	(0)
Pancreas	(49)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(0)	(0)	(1)
Squamous cell papilloma	1 (100%)			1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant	1 (2%)			1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Adrenal medulla	(48)	(50)	(49)	(50)
Pheochromocytoma benign		2 (4%)		
Pheochromocytoma malignant			1 (2%)	
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma		1 (2%)		
Parathyroid gland	(49)	(49)	(48)	(46)
Pituitary gland	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Pars distalis, adenoma	25 (50%)	20 (40%)	16 (32%)	16 (32%)
Pars distalis, carcinoma		3 (6%)	2 (4%)	1 (2%)
Pars distalis, sarcoma, metastatic, skin	1 (2%)			
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	4 (8%)	4 (8%)	3 (6%)	3 (6%)
C-cell, adenoma, multiple				1 (2%)
C-cell, carcinoma	1 (2%)	1 (2%)		
General Body System				
Tissue NOS	(1)	(0)	(0)	(0)

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Genital System				
Clitoral gland	(50)	(50)	(49)	(50)
Adenoma	5 (10%)	9 (18%)	4 (8%)	3 (6%)
Carcinoma	3 (6%)	3 (6%)	1 (2%)	
Carcinoma, multiple	1 (2%)			
Fibrosarcoma				1 (2%)
Ovary	(50)	(50)	(50)	(49)
Granulosa-theca tumor benign		1 (2%)		
Tubulostromal adenoma			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Adenocarcinoma				1 (2%)
Adenoma				1 (2%)
Fibroma			1 (2%)	
Leiomyoma			1 (2%)	
Leiomyosarcoma	1 (2%)			
Polyp stromal	12 (24%)	10 (20%)	14 (28%)	9 (18%)
Polyp stromal, multiple	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Mast cell tumor malignant		1 (2%)		
Lymph node	(9)	(7)	(7)	(8)
Deep cervical, carcinoma, metastatic, thyroid gland		1 (14%)		
Mediastinal, carcinoma, metastatic, thyroid gland		1 (14%)		
Lymph node, mandibular	(2)	(3)	(3)	(5)
Lymph node, mesenteric	(49)	(50)	(50)	(49)
Spleen	(49)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Thymus	(49)	(50)	(49)	(49)
Thymoma benign			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	2 (4%)		1 (2%)
Adenoma, multiple	1 (2%)			
Carcinoma	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Carcinoma, multiple	1 (2%)			
Fibroadenoma	22 (44%)	17 (34%)	7 (14%)	16 (32%)
Fibroadenoma, multiple	8 (16%)	3 (6%)	4 (8%)	1 (2%)
Skin	(50)	(49)	(50)	(50)
Neural crest tumor	1 (2%)			
Squamous cell papilloma		1 (2%)		
Trichoepithelioma	1 (2%)			
Subcutaneous tissue, fibroma	1 (2%)	4 (8%)	3 (6%)	1 (2%)
Subcutaneous tissue, fibrosarcoma		2 (4%)		
Subcutaneous tissue, sarcoma	2 (4%)			
Subcutaneous tissue, schwannoma benign		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(0)	(0)	(2)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant		1 (2%)	1 (2%)	
Carcinoma, metastatic, pituitary gland		2 (4%)	1 (2%)	
Ependymoma malignant		1 (2%)		
Granular cell tumor benign			1 (2%)	1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)		
Hemangiosarcoma, metastatic, spleen			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(49)
Zymbal's gland	(0)	(0)	(1)	(0)
Carcinoma			1 (100%)	
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Sarcoma	1 (2%)			
Urinary bladder	(50)	(49)	(50)	(50)
Transitional epithelial carcinoma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Leukemia granulocytic				1 (2%)
Leukemia mononuclear	9 (18%)	9 (18%)	7 (14%)	6 (12%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	47	45	39
Total primary neoplasms	111	104	77	78
Total animals with benign neoplasms	46	44	40	36
Total benign neoplasms	86	80	62	65
Total animals with malignant neoplasms	19	18	13	12
Total malignant neoplasms	24	24	15	13
Total animals with metastatic neoplasms	1	3	2	
Total metastatic neoplasms	1	4	2	
Total animals with uncertain neoplasm–benign or malignant	1			
Total uncertain neoplasms	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 Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate[b]	2.2%	2.2%	6.5%	4.3%
Terminal rate[c]	1/30 (3%)	1/36 (3%)	3/42 (7%)	2/36 (6%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test[d]	P=0.401	P=0.760N	P=0.314	P=0.509
Clitoral Gland: Adenoma				
Overall rate	5/50 (10%)	9/50 (18%)	4/49 (8%)	3/50 (6%)
Adjusted rate	11.1%	19.9%	8.9%	6.5%
Terminal rate	2/30 (7%)	8/36 (22%)	4/41 (10%)	3/36 (8%)
First incidence (days)	663	639	729 (T)	729 (T)
Poly-3 test	P=0.116N	P=0.191	P=0.505N	P=0.347N
Clitoral Gland: Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	1/49 (2%)	0/50 (0%)
Adjusted rate	8.9%	6.6%	2.2%	0.0%
Terminal rate	2/30 (7%)	2/36 (6%)	1/41 (2%)	0/36 (0%)
First incidence (days)	703	653	729 (T)	—[e]
Poly-3 test	P=0.034N	P=0.498N	P=0.179N	P=0.057N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	9/50 (18%)	11/50 (22%)	5/49 (10%)	3/50 (6%)
Adjusted rate	19.8%	24.2%	11.1%	6.5%
Terminal rate	4/30 (13%)	9/36 (25%)	5/41 (12%)	3/36 (8%)
First incidence (days)	663	639	729 (T)	729 (T)
Poly-3 test	P=0.016N	P=0.403	P=0.198N	P=0.056N
Liver: Hepatocellular Adenoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	8/50 (16%)
Adjusted rate	0.0%	0.0%	2.2%	17.2%
Terminal rate	0/30 (0%)	0/36 (0%)	1/42 (2%)	6/36 (17%)
First incidence (days)	—	—	729 (T)	666
Poly-3 test	P<0.001	—[f]	P=0.505	P=0.004
Mammary Gland: Fibroadenoma				
Overall rate	30/50 (60%)	20/50 (40%)	11/50 (22%)	17/50 (34%)
Adjusted rate	64.6%	43.5%	24.0%	36.2%
Terminal rate	21/30 (70%)	17/36 (47%)	11/42 (26%)	13/36 (36%)
First incidence (days)	611	585	729 (T)	568
Poly-3 test	P=0.015N	P=0.029N	P<0.001N	P=0.004N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	30/50 (60%)	21/50 (42%)	11/50 (22%)	18/50 (36%)
Adjusted rate	64.6%	45.3%	24.0%	38.3%
Terminal rate	21/30 (70%)	17/36 (47%)	11/42 (26%)	14/36 (39%)
First incidence (days)	611	585	729 (T)	568
Poly-3 test	P=0.024N	P=0.043N	P<0.001N	P=0.007N
Mammary Gland: Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	8.9%	2.2%	2.2%	4.3%
Terminal rate	3/30 (10%)	0/36 (0%)	0/42 (0%)	0/36 (0%)
First incidence (days)	663	709	565	716
Poly-3 test	P=0.433N	P=0.179N	P=0.170N	P=0.326N

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Mammary Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rate	13.3%	6.6%	2.2%	6.5%
Terminal rate	5/30 (17%)	1/36 (3%)	0/42 (0%)	1/36 (3%)
First incidence (days)	663	627	565	716
Poly-3 test	P=0.277N	P=0.239N	P=0.051N	P=0.230N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	31/50 (62%)	22/50 (44%)	12/50 (24%)	19/50 (38%)
Adjusted rate	66.4%	47.4%	25.8%	40.4%
Terminal rate	21/30 (70%)	17/36 (47%)	11/42 (26%)	14/36 (39%)
First incidence (days)	611	585	565	568
Poly-3 test	P=0.025N	P=0.045N	P<0.001N	P=0.008N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	25/50 (50%)	20/50 (40%)	16/50 (32%)	16/50 (32%)
Adjusted rate	54.0%	43.2%	34.5%	34.5%
Terminal rate	19/30 (63%)	16/36 (44%)	15/42 (36%)	13/36 (36%)
First incidence (days)	537	589	569	687
Poly-3 test	P=0.058N	P=0.198N	P=0.042N	P=0.043N
Pituitary Gland (Pars Distalis): Carcinoma				
Overall rate	0/50 (0%)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted rate	0.0%	8.9%	4.3%	2.2%
Terminal rate	0/30 (0%)	3/36 (8%)	1/42 (2%)	1/36 (3%)
First incidence (days)	—	726	565	729 (T)
Poly-3 test	P=0.460N	P=0.060	P=0.245	P=0.505
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	25/50 (50%)	24/50 (48%)	18/50 (36%)	17/50 (34%)
Adjusted rate	54.0%	51.8%	38.3%	36.7%
Terminal rate	19/30 (63%)	19/36 (53%)	16/42 (38%)	14/36 (39%)
First incidence (days)	537	589	565	687
Poly-3 test	P=0.044N	P=0.497N	P=0.091N	P=0.067N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	1/50 (2%)	4/50 (8%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	8.7%	6.5%	2.2%
Terminal rate	1/30 (3%)	1/36 (3%)	2/42 (5%)	0/36 (0%)
First incidence (days)	729 (T)	568	666	666
Poly-3 test	P=0.331N	P=0.187	P=0.315	P=0.754N
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma				
Overall rate	3/50 (6%)	5/50 (10%)	3/50 (6%)	1/50 (2%)
Adjusted rate	6.6%	10.9%	6.5%	2.2%
Terminal rate	1/30 (3%)	2/36 (6%)	2/42 (5%)	0/36 (0%)
First incidence (days)	575	568	666	666
Poly-3 test	P=0.130N	P=0.360	P=0.658N	P=0.301N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	4/50 (8%)	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted rate	8.9%	8.8%	6.5%	8.7%
Terminal rate	4/30 (13%)	3/36 (8%)	3/42 (7%)	4/36 (11%)
First incidence (days)	729 (T)	635	729 (T)	729 (T)
Poly-3 test	P=0.578N	P=0.638N	P=0.487N	P=0.629N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	5/50 (10%)	5/50 (10%)	3/50 (6%)	4/50 (8%)
Adjusted rate	11.2%	10.9%	6.5%	8.7%
Terminal rate	5/30 (17%)	3/36 (8%)	3/42 (7%)	4/36 (11%)
First incidence (days)	729 (T)	585	729 (T)	729 (T)
Poly-3 test	P=0.414N	P=0.618N	P=0.344N	P=0.483N
Uterus: Stromal Polyp				
Overall rate	14/50 (28%)	11/50 (22%)	16/50 (32%)	10/50 (20%)
Adjusted rate	29.5%	23.8%	34.7%	21.5%
Terminal rate	7/30 (23%)	8/36 (22%)	15/42 (36%)	9/36 (25%)
First incidence (days)	404	568	663	563
Poly-3 test	P=0.274N	P=0.348N	P=0.377	P=0.255N
All Organs: Mononuclear Cell Leukemia				
Overall rate	9/50 (18%)	9/50 (18%)	7/50 (14%)	6/50 (12%)
Adjusted rate	19.7%	19.3%	14.9%	12.7%
Terminal rate	4/30 (13%)	3/36 (8%)	5/42 (12%)	1/36 (3%)
First incidence (days)	611	635	474	563
Poly-3 test	P=0.198N	P=0.581N	P=0.369N	P=0.261N
All Organs: Benign Neoplasms				
Overall rate	46/50 (92%)	44/50 (88%)	40/50 (80%)	36/50 (72%)
Adjusted rate	93.2%	90.4%	84.3%	75.0%
Terminal rate	29/30 (97%)	33/36 (92%)	36/42 (86%)	28/36 (78%)
First incidence (days)	404	568	565	563
Poly-3 test	P=0.003N	P=0.439N	P=0.130N	P=0.010N
All Organs: Malignant Neoplasms				
Overall rate	19/50 (38%)	18/50 (36%)	13/50 (26%)	12/50 (24%)
Adjusted rate	40.6%	37.6%	27.0%	25.2%
Terminal rate	10/30 (33%)	8/36 (22%)	8/42 (19%)	5/36 (14%)
First incidence (days)	575	568	474	563
Poly-3 test	P=0.061N	P=0.467N	P=0.116N	P=0.083N
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	47/50 (94%)	45/50 (90%)	39/50 (78%)
Adjusted rate	97.0%	95.9%	91.8%	80.3%
Terminal rate	29/30 (97%)	34/36 (94%)	38/42 (91%)	28/36 (78%)
First incidence (days)	404	568	474	563
Poly-3 test	P<0.001N	P=0.604N	P=0.246N	P=0.008N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, liver, 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 control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of the statistic cannot be computed

TABLE B3
Historical Incidence of Hepatocellular Adenoma in Control Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Feed Studies	
Benzophenone	0/50
Chromium picolinate monohydrate	2/50
Goldenseal root powder	0/50
4-Methylimidazole	0/50
Milk thistle extract	2/50
Total (%)	4/250 (1.6%)
Mean ± standard deviation	1.6% ± 2.2%
Range	0%-4%
Overall Historical Incidence: All Routes	
Total (%)	16/1,350 (1.2%)
Mean ± standard deviation	1.2% ± 2.6%
Range	0%-12%

[a] Data as of November 17, 2008

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	14	9	5	12
Natural deaths	6	5	3	2
Survivors				
Terminal sacrifice	30	36	42	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(48)	(50)	(50)	(49)
Edema	1 (2%)			2 (4%)
Intestine small, jejunum	(46)	(47)	(48)	(50)
Epithelium, hyperplasia				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	2 (4%)	
Basophilic focus	44 (88%)	44 (88%)	46 (92%)	27 (54%)
Clear cell focus	4 (8%)	5 (10%)	10 (20%)	2 (4%)
Cyst			1 (2%)	1 (2%)
Eosinophilic focus	2 (4%)	24 (48%)	29 (58%)	22 (44%)
Hematopoietic cell proliferation	1 (2%)			
Hemorrhage		1 (2%)	1 (2%)	1 (2%)
Hepatodiaphragmatic nodule	7 (14%)	17 (34%)	8 (16%)	9 (18%)
Infiltration cellular, mixed cell	7 (14%)	7 (14%)	9 (18%)	11 (22%)
Mixed cell focus	6 (12%)	5 (10%)	13 (26%)	9 (18%)
Necrosis, focal	1 (2%)			1 (2%)
Bile duct, hyperplasia	2 (4%)	4 (8%)	9 (18%)	3 (6%)
Centrilobular, necrosis	1 (2%)		1 (2%)	
Hepatocyte, degeneration	1 (2%)	2 (4%)	12 (24%)	24 (48%)
Hepatocyte, hypertrophy	2 (4%)	10 (20%)	27 (54%)	38 (76%)
Hepatocyte, vacuolization cytoplasmic	4 (8%)	4 (8%)	1 (2%)	3 (6%)
Kupffer cell, pigmentation	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Mesentery	(9)	(7)	(4)	(2)
Accessory spleen		2 (29%)	2 (50%)	1 (50%)
Fat, necrosis	9 (100%)	5 (71%)	3 (75%)	1 (50%)
Oral mucosa	(0)	(0)	(1)	(0)
Ulcer			1 (100%)	
Pancreas	(49)	(50)	(50)	(50)
Atrophy	10 (20%)	17 (34%)	17 (34%)	14 (28%)
Cyst	4 (8%)	10 (20%)	6 (12%)	13 (26%)
Acinus, cytoplasmic alteration	1 (2%)	1 (2%)		1 (2%)
Acinus, hyperplasia, focal		1 (2%)	1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	7 (14%)		5 (10%)	3 (6%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema		1 (2%)	1 (2%)	2 (4%)
Ulcer	2 (4%)	2 (4%)		3 (6%)
Epithelium, hyperplasia		2 (4%)	1 (2%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Edema		1 (2%)		
Erosion	4 (8%)	2 (4%)	1 (2%)	2 (4%)
Ulcer				1 (2%)
Tongue	(1)	(0)	(0)	(1)
Inflammation, granulomatous				1 (100%)

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

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	24 (48%)	24 (48%)	21 (42%)	15 (30%)
Inflammation, chronic	1 (2%)			
Thrombosis	1 (2%)	1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	3 (6%)	3 (6%)	9 (18%)	6 (12%)
Angiectasis			2 (4%)	1 (2%)
Degeneration, fatty	9 (18%)	15 (30%)	11 (22%)	11 (22%)
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia, focal	5 (10%)	6 (12%)	4 (8%)	5 (10%)
Hypertrophy, focal	1 (2%)	2 (4%)	6 (12%)	5 (10%)
Necrosis	1 (2%)	1 (2%)		2 (4%)
Adrenal medulla	(48)	(50)	(49)	(50)
Hyperplasia	1 (2%)	2 (4%)	5 (10%)	7 (14%)
Infiltration cellular, mononuclear cell		1 (2%)		
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia			1 (2%)	
Parathyroid gland	(49)	(49)	(48)	(46)
Hyperplasia		1 (2%)		
Pituitary gland	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell		1 (2%)		
Pigmentation	1 (2%)			
Pars distalis, angiectasis	8 (16%)	7 (14%)	3 (6%)	8 (16%)
Pars distalis, cyst	30 (60%)	23 (46%)	27 (54%)	20 (40%)
Pars distalis, hyperplasia, focal	13 (26%)	4 (8%)	12 (24%)	9 (18%)
Pars intermedia, angiectasis	1 (2%)	1 (2%)		2 (4%)
Pars intermedia, cyst	3 (6%)			2 (4%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	17 (34%)	5 (10%)	6 (12%)	5 (10%)
Follicle, cyst		2 (4%)	2 (4%)	1 (2%)
Follicular cell, hyperplasia		2 (4%)		
General Body System				
Tissue NOS	(1)	(0)	(0)	(0)
Genital System				
Clitoral gland	(50)	(50)	(49)	(50)
Cyst	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Hyperplasia	1 (2%)	2 (4%)		
Hyperplasia, focal	1 (2%)	5 (10%)	6 (12%)	
Inflammation, chronic		4 (8%)	2 (4%)	2 (4%)
Ovary	(50)	(50)	(50)	(49)
Cyst	8 (16%)	5 (10%)	14 (28%)	8 (16%)
Corpus luteum, vacuolization cytoplasmic			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Decidual reaction		1 (2%)		
Hemorrhage	1 (2%)			
Hyperplasia, cystic	8 (16%)	9 (18%)	14 (28%)	13 (26%)
Inflammation, suppurative			1 (2%)	
Cervix, cyst, squamous				1 (2%)
Myometrium, hypertrophy			1 (2%)	

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	10 (20%)	3 (6%)	5 (10%)	6 (12%)
Infiltration cellular, histiocyte		1 (2%)	1 (2%)	3 (6%)
Infiltration cellular, mixed cell			1 (2%)	
Myelofibrosis	1 (2%)	1 (2%)		5 (10%)
Lymph node	(9)	(7)	(7)	(8)
Hemorrhage	1 (11%)			
Pigmentation	1 (11%)			
Deep cervical, ectasia			1 (14%)	
Mediastinal, hematopoietic cell proliferation	1 (11%)			
Mediastinal, hemorrhage	5 (56%)	2 (29%)	4 (57%)	1 (13%)
Mediastinal, hyperplasia, lymphoid	4 (44%)	3 (43%)	1 (14%)	2 (25%)
Mediastinal, pigmentation	2 (22%)	3 (43%)	2 (29%)	1 (13%)
Pancreatic, hemorrhage	2 (22%)	2 (29%)		1 (13%)
Pancreatic, pigmentation		1 (14%)		
Lymph node, mandibular	(2)	(3)	(3)	(5)
Ectasia		1 (33%)		
Hyperplasia, lymphoid		1 (33%)		
Lymph node, mesenteric	(49)	(50)	(50)	(49)
Ectasia				1 (2%)
Hemorrhage	3 (6%)	1 (2%)	3 (6%)	3 (6%)
Hyperplasia, lymphoid	15 (31%)	8 (16%)	10 (20%)	9 (18%)
Spleen	(49)	(50)	(50)	(50)
Accessory spleen	2 (4%)			
Fibrosis		1 (2%)		2 (4%)
Hematopoietic cell proliferation	37 (76%)	34 (68%)	42 (84%)	35 (70%)
Hemorrhage				1 (2%)
Infiltration cellular, mixed cell				3 (6%)
Necrosis				1 (2%)
Lymphoid follicle, atrophy				1 (2%)
Thymus	(49)	(50)	(49)	(49)
Atrophy		1 (2%)		1 (2%)
Cyst	1 (2%)		1 (2%)	
Hyperplasia, lymphoid			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	49 (98%)	46 (92%)	45 (90%)	34 (68%)
Skin	(50)	(49)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	
Edema				1 (2%)
Hemorrhage	1 (2%)			
Hyperkeratosis	1 (2%)		1 (2%)	1 (2%)
Inflammation, chronic			1 (2%)	
Ulcer	1 (2%)			
Epidermis, hyperplasia	1 (2%)		1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fracture	1 (2%)			
Osteopetrosis		1 (2%)		
Skeletal muscle	(0)	(0)	(0)	(2)
Hemorrhage				1 (50%)
Inflammation, suppurative				1 (50%)

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	9 (18%)	8 (16%)	3 (6%)	8 (16%)
Gliosis	1 (2%)			
Hemorrhage	2 (4%)		1 (2%)	2 (4%)
Necrosis	1 (2%)			1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Edema	1 (2%)	1 (2%)		
Foreign body		1 (2%)	1 (2%)	
Hemorrhage	6 (12%)		2 (4%)	10 (20%)
Hyperplasia, lymphoid	1 (2%)			
Infiltration cellular, histiocyte	39 (78%)	34 (68%)	37 (74%)	39 (78%)
Inflammation, chronic	18 (36%)	21 (42%)	19 (38%)	14 (28%)
Metaplasia, osseous		2 (4%)	2 (4%)	2 (4%)
Alveolar epithelium, hyperplasia	7 (14%)	7 (14%)	5 (10%)	6 (12%)
Serosa, hyperplasia		1 (2%)	1 (2%)	1 (2%)
Nose	(50)	(50)	(50)	(50)
Foreign body	4 (8%)	3 (6%)	2 (4%)	2 (4%)
Inflammation, chronic	2 (4%)		1 (2%)	2 (4%)
Respiratory epithelium, hyperplasia	1 (2%)			1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	4 (8%)	2 (4%)	1 (2%)	2 (4%)
Retina, degeneration	4 (8%)	2 (4%)	1 (2%)	2 (4%)
Harderian gland	(50)	(50)	(50)	(49)
Hyperplasia, focal	2 (4%)			
Inflammation, chronic				2 (4%)
Zymbal's gland	(0)	(0)	(1)	(0)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Cyst	1 (2%)		1 (2%)	
Glomerulosclerosis				1 (2%)
Infarct		1 (2%)		
Infiltration cellular, mononuclear cell		1 (2%)		
Inflammation, suppurative	1 (2%)		1 (2%)	
Metaplasia, osseous	1 (2%)			
Nephropathy	36 (73%)	24 (48%)	33 (66%)	45 (90%)
Papilla, necrosis	1 (2%)			
Renal tubule, accumulation, hyaline droplet	2 (4%)		1 (2%)	1 (2%)
Renal tubule, necrosis	1 (2%)			1 (2%)
Renal tubule, pigmentation	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Transitional epithelium, hyperplasia	2 (4%)		2 (4%)	
Urinary bladder	(50)	(49)	(50)	(50)
Edema	1 (2%)			
Transitional epithelium, hyperplasia	1 (2%)			

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF GOLDENSEAL ROOT POWDER

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TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund		3	2	3
Natural deaths	7	9	3	2
Survivors				
Died last week of study		1		
Terminal sacrifice	43	37	45	45
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(47)	(46)	(48)	(50)
Hemangiosarcoma			1 (2%)	
Intestine small, duodenum	(44)	(43)	(47)	(50)
Adenoma		2 (5%)		
Intestine small, jejunum	(46)	(43)	(47)	(48)
Carcinoma	1 (2%)	1 (2%)		
Carcinoma, multiple	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Chemodectoma, malignant, metastatic, uncertain primary site			1 (2%)	
Hemangiosarcoma		2 (4%)	2 (4%)	1 (2%)
Hemangiosarcoma, metastatic, spleen	1 (2%)	1 (2%)		1 (2%)
Hemangiosarcoma, multiple				1 (2%)
Hepatoblastoma	1 (2%)	2 (4%)	1 (2%)	4 (8%)
Hepatoblastoma, multiple				2 (4%)
Hepatocellular adenoma	19 (38%)	11 (22%)	12 (24%)	11 (22%)
Hepatocellular adenoma, multiple	3 (6%)	5 (10%)	11 (22%)	18 (36%)
Hepatocellular carcinoma	6 (12%)	10 (20%)	11 (22%)	10 (20%)
Hepatocellular carcinoma, multiple	2 (4%)	4 (8%)	4 (8%)	2 (4%)
Hepatocholangiocarcinoma	1 (2%)		1 (2%)	
Sarcoma, metastatic, pancreas			1 (2%)	
Mesentery	(6)	(3)	(12)	(1)
Hepatoblastoma, metastatic, liver				1 (100%)
Hepatocholangiocarcinoma, metastatic, liver	1 (17%)		1 (8%)	
Sarcoma, metastatic, pancreas			1 (8%)	
Pancreas	(49)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Sarcoma			1 (2%)	
Acinus, adenoma	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Squamous cell papilloma		2 (4%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver			1` (2%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver		1 (2%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			1 (2%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Capsule, adenoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Extra adrenal tissue, hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Extra adrenal tissue, sarcoma, metastatic, pancreas			1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(49)
Pheochromocytoma, benign			1 (2%)	1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Parathyroid gland	(45)	(45)	(47)	(49)
Pituitary gland	(50)	(49)	(49)	(50)
Pars intermedia, adenoma				1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	1 (2%)		1 (2%)	
General Body System				
None				
Genital System				
Coagulating gland		(1)		
Epididymis	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)		1 (2%)	
Sarcoma, metastatic, pancreas			1 (2%)	
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma			1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Hemangiosarcoma, metastatic, spleen				1 (2%)
Lymph node	(2)	(5)	(2)	(2)
Sarcoma, metastatic, pancreas			1 (50%)	
Bronchial, hemangiosarcoma, metastatic, spleen		1 (20%)		
Inguinal, hemangiosarcoma				1 (50%)
Mediastinal, hepatocholangiocarcinoma, metastatic, liver	1 (50%)			
Renal, hepatoblastoma, metastatic, liver		1 (20%)		
Lymph node, mandibular	(46)	(46)	(50)	(49)
Lymph node, mesenteric	(48)	(44)	(49)	(48)
Hemangiosarcoma, metastatic, spleen		1 (2%)		
Hepatoblastoma, metastatic, liver		1 (2%)		

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Hematopoietic System (continued)				
Spleen	(50)	(49)	(50)	(50)
Hemangioma		1 (2%)		
Hemangiosarcoma	1 (2%)	3 (6%)		1 (2%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Thymus	(44)	(39)	(43)	(41)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			
Lipoma			1 (2%)	
Subcutaneous tissue, hemangioma		1 (2%)		
Subcutaneous tissue, sarcoma				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle		(1)	(3)	
Hepatocholangiocarcinoma, metastatic, liver			1 (33%)	
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar, adenoma	4 (8%)	5 (10%)	7 (14%)	8 (16%)
Alveolar/bronchiolar, adenoma, multiple			2 (4%)	
Alveolar/bronchiolar, carcinoma	6 (12%)	4 (8%)	3 (6%)	
Alveolar/bronchiolar, carcinoma, multiple			1 (2%)	
Hemangiosarcoma, metastatic, spleen		1 (2%)		
Hepatoblastoma, metastatic, liver		2 (4%)		1 (2%)
Hepatocellular carcinoma, metastatic, liver	2 (4%)	3 (6%)	4 (8%)	2 (4%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)		1 (2%)	
Sarcoma, metastatic, pancreas			1 (2%)	
Sarcoma, metastatic, skin				1 (2%)
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(46)	(50)	(49)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	7 (14%)	11 (22%)	3 (6%)	6 (12%)
Carcinoma	2 (4%)	3 (6%)	2 (4%)	2 (4%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Capsule, hepatocholangiocarcinoma, metastatic, liver	1 (2%)			
Urinary bladder	(49)	(50)	(50)	(50)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		2 (4%)	2 (4%)	
Lymphoma, malignant	1 (2%)	2 (4%)	1 (2%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	38	42	43	40
Total primary neoplasms	60	74	70	72
Total animals with benign neoplasms	28	27	33	36
Total benign neoplasms	37	40	40	47
Total animals with malignant neoplasms	20	26	25	19
Total malignant neoplasms	23	34	30	25
Total animals with metastatic neoplasms	4	6	6	5
Total metastatic neoplasms	11	14	22	7
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 Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Harderian Gland: Adenoma				
Overall rate[a]	7/50 (14%)	11/50 (22%)	3/50 (6%)	6/50 (12%)
Adjusted rate[b]	14.7%	23.1%	6.3%	12.3%
Terminal rate[c]	7/43 (16%)	8/38 (21%)	3/45 (7%)	5/45 (11%)
First incidence (days)	729 (T)	557	729 (T)	722
Poly-3 test[d]	P=0.230N	P=0.217	P=0.158N	P=0.479N
Harderian Gland: Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	4.2%	6.4%	4.2%	4.1%
Terminal rate	2/43 (5%)	2/38 (5%)	2/45 (4%)	2/45 (4%)
First incidence (days)	729 (T)	625	729 (T)	729 (T)
Poly-3 test	P=0.510N	P=0.492	P=0.693N	P=0.684N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	9/50 (18%)	13/50 (26%)	4/50 (8%)	8/50 (16%)
Adjusted rate	18.9%	27.1%	8.4%	16.3%
Terminal rate	9/43 (21%)	9/38 (24%)	4/45 (9%)	7/45 (16%)
First incidence (days)	729 (T)	557	729 (T)	722
Poly-3 test	P=0.249N	P=0.240	P=0.115N	P=0.475N
Small Intestine (Duodenum or Jejunum): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	4.2%	6.5%	0.0%	0.0%
Terminal rate	1/43 (2%)	3/38 (8%)	0/45 (0%)	0/45 (0%)
First incidence (days)	632	729 (T)	—[e]	—
Poly-3 test	P=0.082N	P=0.485	P=0.239N	P=0.233N
Liver: Hepatocellular Adenoma				
Overall rate	22/50 (44%)	16/50 (32%)	23/50 (46%)	29/50 (58%)
Adjusted rate	45.7%	33.8%	48.0%	58.2%
Terminal rate	19/43 (44%)	13/38 (34%)	22/45 (49%)	26/45 (58%)
First incidence (days)	677	555	666	596
Poly-3 test	P=0.030	P=0.162N	P=0.492	P=0.150
Liver: Hepatocellular Carcinoma				
Overall rate	8/50 (16%)	14/50 (28%)	15/50 (30%)	12/50 (24%)
Adjusted rate	16.7%	28.7%	30.3%	24.2%
Terminal rate	6/43 (14%)	8/38 (21%)	11/45 (24%)	8/45 (18%)
First incidence (days)	681	555	551	596
Poly-3 test	P=0.436	P=0.120	P=0.088	P=0.254
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	26/50 (52%)	27/50 (54%)	31/50 (62%)	33/50 (66%)
Adjusted rate	54.0%	54.9%	62.7%	66.0%
Terminal rate	23/43 (54%)	19/38 (50%)	27/45 (60%)	28/45 (62%)
First incidence (days)	677	555	551	596
Poly-3 test	P=0.111	P=0.544	P=0.252	P=0.156
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)	6/50 (12%)
Adjusted rate	2.1%	4.3%	2.1%	12.3%
Terminal rate	1/43 (2%)	1/38 (3%)	1/45 (2%)	5/45 (11%)
First incidence (days)	729 (T)	673	729 (T)	722
Poly-3 test	P=0.016	P=0.492	P=0.760N	P=0.061

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	9/50 (18%)	15/50 (30%)	15/50 (30%)	15/50 (30%)
Adjusted rate	18.8%	30.6%	30.3%	30.2%
Terminal rate	7/43 (16%)	8/38 (21%)	11/45 (24%)	11/45 (24%)
First incidence (days)	681	555	551	596
Poly-3 test	P=0.248	P=0.131	P=0.138	P=0.141
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	26/50 (52%)	28/50 (56%)	31/50 (62%)	33/50 (66%)
Adjusted rate	54.0%	56.7%	62.7%	66.0%
Terminal rate	23/43 (54%)	19/38 (50%)	27/45 (60%)	28/45 (62%)
First incidence (days)	677	555	551	596
Poly-3 test	P=0.128	P=0.473	P=0.252	P=0.156
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	5/50 (10%)	9/50 (18%)	8/50 (16%)
Adjusted rate	8.4%	10.8%	18.9%	16.2%
Terminal rate	4/43 (9%)	5/38 (13%)	9/45 (20%)	7/45 (16%)
First incidence (days)	729 (T)	729 (T)	729 (T)	631
Poly-3 test	P=0.178	P=0.483	P=0.116	P=0.195
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	6/50 (12%)	4/50 (8%)	4/50 (8%)	0/50 (0%)
Adjusted rate	12.5%	8.6%	8.4%	0.0%
Terminal rate	5/43 (12%)	3/38 (8%)	4/45 (9%)	0/45 (0%)
First incidence (days)	664	709	729 (T)	—
Poly-3 test	P=0.014N	P=0.389N	P=0.373N	P=0.014N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	10/50 (20%)	9/50 (18%)	12/50 (24%)	8/50 (16%)
Adjusted rate	20.9%	19.4%	25.2%	16.2%
Terminal rate	9/43 (21%)	8/38 (21%)	12/45 (27%)	7/45 (16%)
First incidence (days)	664	709	729 (T)	631
Poly-3 test	P=0.325N	P=0.529N	P=0.400	P=0.372N
Spleen: Hemangiosarcoma				
Overall rate	1/50 (2%)	3/49 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.1%	6.5%	0.0%	2.0%
Terminal rate	1/43 (2%)	2/38 (5%)	0/45 (0%)	0/45 (0%)
First incidence (days)	729 (T)	680	—	710
Poly-3 test	P=0.412N	P=0.293	P=0.500N	P=0.754N
All Organs: Hemangiosarcoma				
Overall rate	2/50 (4%)	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted rate	4.2%	8.6%	6.3%	8.2%
Terminal rate	2/43 (5%)	3/38 (8%)	3/45 (7%)	3/45 (7%)
First incidence (days)	729 (T)	680	729 (T)	710
Poly-3 test	P=0.396	P=0.327	P=0.500	P=0.351
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	6/50 (12%)	3/50 (6%)	4/50 (8%)
Adjusted rate	4.2%	12.8%	6.3%	8.2%
Terminal rate	2/43 (5%)	4/38 (11%)	3/45 (7%)	3/45 (7%)
First incidence (days)	729 (T)	636	729 (T)	710
Poly-3 test	P=0.540	P=0.128	P=0.500	P=0.351

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
All Organs: Benign Neoplasms				
Overall rate	28/50 (56%)	27/50 (54%)	33/50 (66%)	36/50 (72%)
Adjusted rate	58.2%	55.9%	68.8%	72.2%
Terminal rate	25/43 (58%)	22/38 (58%)	32/45 (71%)	32/45 (71%)
First incidence (days)	677	555	666	596
Poly-3 test	P=0.048	P=0.493N	P=0.188	P=0.104
All Organs: Malignant Neoplasms				
Overall rate	20/50 (40%)	26/50 (52%)	26/50 (52%)	19/50 (38%)
Adjusted rate	40.7%	52.8%	52.0%	38.0%
Terminal rate	15/43 (35%)	16/38 (42%)	21/45 (47%)	14/45 (31%)
First incidence (days)	550	555	551	596
Poly-3 test	P=0.217N	P=0.160	P=0.178	P=0.472N
All Organs: Benign or Malignant Neoplasms				
Overall rate	38/50 (76%)	42/50 (84%)	43/50 (86%)	40/50 (80%)
Adjusted rate	77.1%	84.0%	86.0%	80.0%
Terminal rate	32/43 (74%)	30/38 (79%)	38/45 (84%)	35/45 (78%)
First incidence (days)	550	555	551	596
Poly-3 test	P=0.559N	P=0.268	P=0.186	P=0.455

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and spleen; 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 control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE C3
Historical Incidence of Liver Neoplasms in Control Male B6C3F1 Mice[a]

Study	Incidence in Controls			
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Feed Studies				
Benzophenone	11/50	8/50	0/50	18/50
Chromium picolinate monohydrate	21/50	15/50	0/50	32/50
Goldenseal root powder	22/50	8/50	1/50	26/50
4-Methylimidazole	17/50	10/50	0/50	23/50
Milk thistle extract	12/50	17/50	0/50	26/50
Total (%)	83/250 (33.2%)	58/250 (23.2%)	1/250 (0.4%)	125/250 (50.0%)
Mean ± standard deviation	33.2% ± 10.1%	23.2% ± 8.3%	0.4% ± 0.9%	50.0% ± 10.2%
Range	22%-44%	16%-34%	0%-2%	36%-64%
Overall Historical Incidence: All Routes				
Total (%)	733/1,447 (50.7%)	415/1,447 (28.7%)	48/1,447 (3.3%)	972/1,447 (67.2%)
Mean ± standard deviation	50.7% ± 13.9%	28.7% ± 8.8%	3.3% ± 6.4%	67.2% ± 13.1%
Range	22%-72%	16%-52%	0%-34%	36%-92%

[a] Data as of November 19, 2008

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund		3	2	3
Natural deaths	7	9	3	2
Survivors				
Died last week of study		1		
Terminal sacrifice	43	37	45	45
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(47)	(46)	(48)	(50)
Intestine small, duodenum	(44)	(43)	(47)	(50)
Intestine small, jejunum	(46)	(43)	(47)	(48)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Epithelium, hyperplasia				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Basophilic focus	1 (2%)	3 (6%)	3 (6%)	3 (6%)
Clear cell focus	10 (20%)	10 (20%)	11 (22%)	12 (24%)
Cyst		1 (2%)		1 (2%)
Eosinophilic focus	7 (14%)	14 (28%)	14 (28%)	24 (48%)
Fibrosis			1 (2%)	
Hematopoietic cell proliferation		1 (2%)	1 (2%)	
Hemorrhage		1 (2%)		
Hepatodiaphragmatic nodule	1 (2%)	1 (2%)		
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)	4 (8%)	3 (6%)
Infiltration cellular, mixed cell	3 (6%)	6 (12%)	3 (6%)	4 (8%)
Mixed cell focus	1 (2%)	7 (14%)	9 (18%)	6 (12%)
Necrosis, focal	2 (4%)	3 (6%)	5 (10%)	6 (12%)
Tension lipidosis		1 (2%)		
Bile duct, hyperplasia		1 (2%)		
Hepatocyte, hyperplasia, focal			1 (2%)	
Hepatocyte, hypertrophy			1 (2%)	
Hepatocyte, vacuolization cytoplasmic	6 (12%)	11 (22%)	15 (30%)	7 (14%)
Hepatocyte, vacuolization cytoplasmic, focal			1 (2%)	
Kupffer cell, pigmentation	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Mesentery	(6)	(3)	(12)	(1)
Fibrosis	1 (17%)		1 (8%)	1 (100%)
Inflammation, suppurative		1 (33%)	1 (8%)	
Fat, necrosis	5 (83%)	1 (33%)	6 (50%)	
Pancreas	(49)	(50)	(50)	(50)
Atrophy			1 (2%)	
Cyst			1 (2%)	
Infiltration cellular, lymphocyte				1 (2%)
Acinus, cytoplasmic alteration	2 (4%)	5 (10%)		2 (4%)
Acinus, hyperplasia, focal	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)			
Ulcer	1 (2%)	2 (4%)		
Epithelium, hyperplasia	3 (6%)	5 (10%)	3 (6%)	2 (4%)

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

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Alimentary System (continued)				
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst		5 (10%)	3 (6%)	1 (2%)
Edema		1 (2%)		
Erosion	1 (2%)			4 (8%)
Epithelium, hyperplasia				1 (2%)
Glands, cyst	1 (2%)			
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	45 (90%)	42 (84%)	44 (88%)	44 (88%)
Thrombosis	1 (2%)			
Artery, inflammation, chronic	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule		1 (2%)	1 (2%)	
Hypertrophy			3 (6%)	
Hypertrophy, focal	13 (26%)	18 (36%)	10 (20%)	5 (10%)
Capsule, hyperplasia	39 (78%)	37 (74%)	41 (82%)	37 (74%)
Adrenal medulla	(50)	(50)	(50)	(49)
Hyperplasia			1 (2%)	
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia	4 (8%)	12 (24%)	11 (22%)	4 (8%)
Parathyroid gland	(45)	(45)	(47)	(49)
Cyst			3 (6%)	
Pituitary gland	(50)	(49)	(49)	(50)
Pars distalis, cyst	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Pars distalis, hyperplasia, focal	2 (4%)			1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicle, cyst		2 (4%)	4 (8%)	
Follicle, degeneration, focal	5 (10%)	6 (12%)	6 (12%)	4 (8%)
Follicular cell, hyperplasia				1 (2%)
General Body System				
None				
Genital System				
Coagulating gland		(1)		
Inflammation, suppurative		1 (100%)		
Epididymis	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Granuloma sperm			1 (2%)	
Preputial gland	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Cyst	19 (38%)	17 (34%)	25 (50%)	13 (26%)
Hyperplasia		1 (2%)	4 (8%)	2 (4%)
Inflammation, suppurative	1 (2%)	3 (6%)	4 (8%)	2 (4%)
Inflammation, chronic	1 (2%)	4 (8%)	3 (6%)	2 (4%)
Bilateral, cyst			1 (2%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Genital System (continued)				
Prostate	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)		
Inflammation, granulomatous			1 (2%)	
Inflammation, chronic		2 (4%)		
Epithelium, hyperplasia	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)		
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, atrophy	1 (2%)		5 (10%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Depletion cellular		1 (2%)		
Hyperplasia	6 (12%)	17 (34%)	14 (28%)	14 (28%)
Lymph node	(2)	(5)	(2)	(2)
Bronchial, hyperplasia				1 (50%)
Iliac, hemorrhage	1 (50%)			
Pancreatic, hemorrhage		1 (20%)		
Renal, hemorrhage		1 (20%)		
Lymph node, mandibular	(46)	(46)	(50)	(49)
Atrophy			1 (2%)	
Hyperplasia, lymphoid		3 (7%)	2 (4%)	2 (4%)
Lymph node, mesenteric	(48)	(44)	(49)	(48)
Atrophy		1 (2%)		
Hemorrhage		1 (2%)		
Hyperplasia, histiocytic	2 (4%)		1 (2%)	1 (2%)
Hyperplasia, lymphoid	3 (6%)	2 (5%)	2 (4%)	2 (4%)
Hyperplasia, plasma cell			1 (2%)	
Spleen	(50)	(49)	(50)	(50)
Atrophy		2 (4%)	1 (2%)	
Hematopoietic cell proliferation	23 (46%)	33 (67%)	27 (54%)	20 (40%)
Hyperplasia	2 (4%)			1 (2%)
Lymphoid follicle, hyperplasia	4 (8%)	3 (6%)	4 (8%)	3 (6%)
Thymus	(44)	(39)	(43)	(41)
Atrophy	3 (7%)	8 (21%)	1 (2%)	2 (5%)
Cyst			2 (5%)	
Hyperplasia, lymphoid		1 (3%)		
Necrosis, lymphoid				1 (2%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	
Inflammation, chronic	1 (2%)	1 (2%)		
Ulcer		1 (2%)		
Epidermis, hyperplasia	1 (2%)	1 (2%)		
Hair follicle, cyst	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, hyperostosis	1 (2%)			
Maxilla, fibrosis		1 (2%)		
Skeletal muscle		(1)	(3)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Edema		1 (2%)		
Fibrosis	1 (2%)			
Infiltration, cellular, histiocyte	2 (4%)		1 (2%)	2 (4%)
Infiltration, cellular, lymphocyte	3 (6%)			1 (2%)
Alveolar epithelium, hyperplasia	6 (12%)	2 (4%)	3 (6%)	5 (10%)
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative			1 (2%)	
Glands, degeneration, cystic		1 (2%)		
Nasolacrimal duct, inflammation, suppurative	1 (2%)			
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(46)	(50)	(49)	(50)
Cataract			1 (2%)	
Inflammation, acute	1 (2%)			
Cornea, inflammation, chronic		1 (2%)		
Harderian gland	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia	1 (2%)			
Hyperplasia, focal		1 (2%)		1 (2%)
Infiltration cellular, lymphocyte			1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	4 (8%)	3 (6%)	9 (18%)	2 (4%)
Glomerulosclerosis			1 (2%)	
Hydronephrosis		1 (2%)		
Infarct	2 (4%)	4 (8%)		1 (2%)
Infiltration cellular, lymphocyte	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Inflammation, suppurative	1 (2%)	1 (2%)		
Metaplasia, osseous	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Nephropathy	42 (84%)	43 (86%)	46 (92%)	48 (96%)
Thrombosis	1 (2%)			
Bilateral, hydronephrosis		1 (2%)		
Papilla, inflammation, suppurative			1 (2%)	
Papilla, necrosis		1 (2%)		
Renal tubule, accumulation, hyaline droplet			1 (2%)	
Renal tubule, pigmentation				2 (4%)
Urinary bladder	(49)	(50)	(50)	(50)
Calculus gross observation		1 (2%)		
Hemorrhage				1 (2%)
Infiltration cellular, lymphocyte		1 (2%)	1 (2%)	1 (2%)
Inflammation, chronic		1 (2%)		
Transitional epithelium, hyperplasia		1 (2%)		

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF GOLDENSEAL ROOT POWDER

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TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	2	2	3
Natural deaths	1	4	12	4
Survivors				
Died last week of study	1			
Terminal sacrifice	44	44	36	43
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(48)	(46)	(39)	(47)
Intestine large, cecum	(48)	(46)	(41)	(47)
Intestine large, rectum	(48)	(47)	(43)	(47)
Leiomyosarcoma			1 (2%)	
Intestine small, ileum	(48)	(46)	(39)	(47)
Intestine small, jejunum	(48)	(46)	(43)	(48)
Liver	(50)	(50)	(49)	(50)
Hepatocellular adenoma	3 (6%)	6 (12%)	6 (12%)	5 (10%)
Hepatocellular adenoma, multiple			1 (2%)	2 (4%)
Hepatocellular carcinoma	3 (6%)	3 (6%)	1 (2%)	1 (2%)
Mesentery	(10)	(6)	(9)	(2)
Pancreas	(48)	(48)	(47)	(50)
Acinus, adenoma			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(48)	(50)
Stomach, glandular	(49)	(49)	(48)	(50)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(48)	(50)
Adenoma		1 (2%)		
Capsule, adenoma		1 (2%)		1 (2%)
Adrenal medulla	(50)	(50)	(49)	(50)
Pheochromocytoma, benign				1 (2%)
Islets, pancreatic	(48)	(49)	(48)	(50)
Adenoma	1 (2%)	1 (2%)		
Parathyroid gland	(45)	(44)	(48)	(47)
Pituitary gland	(50)	(49)	(49)	(49)
Pars distalis, adenoma		2 (4%)	2 (4%)	1 (2%)
Pars intermedia, adenoma				1 (2%)
Thyroid gland	(50)	(50)	(48)	(50)
Follicular cell, adenoma		2 (4%)		
General Body System				
None				

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Genital System				
Clitoral gland	(50)	(50)	(49)	(50)
Ovary	(49)	(48)	(50)	(50)
Cystadenoma	3 (6%)	2 (4%)	1 (2%)	
Granulosa cell tumor benign				1 (2%)
Uterus	(50)	(49)	(50)	(50)
Polyp stromal	2 (4%)	1 (2%)		1 (2%)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, spleen	1 (2%)			
Lymph node	(6)	(4)	(5)	(4)
Iliac, sarcoma	1 (17%)			
Lymph node, mandibular	(50)	(49)	(48)	(49)
Lymph node, mesenteric	(48)	(43)	(48)	(49)
Spleen	(48)	(48)	(47)	(49)
Hemangiosarcoma	1 (2%)		1 (2%)	
Thymus	(49)	(48)	(50)	(48)
Integumentary System				
Mammary gland	(49)	(49)	(48)	(50)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma		1 (2%)		
Sarcoma			1 (2%)	1 (2%)
Trichoepithelioma	1 (2%)			
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, melanoma malignant				1 (2%)
Subcutaneous tissue, sarcoma	2 (4%)	1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(1)	(2)	
Rhabdomyosarcoma			1 (50%)	
Sarcoma	1 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar, adenoma	4 (8%)	2 (4%)	5 (10%)	1 (2%)
Alveolar/bronchiolar, carcinoma	1 (2%)	1 (2%)		1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Sarcoma, metastatic, skeletal muscle			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(49)	(50)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Special Senses System				
Eye	(49)	(47)	(41)	(46)
Harderian gland	(50)	(49)	(50)	(50)
Adenoma	4 (8%)	1 (2%)	1 (2%)	3 (6%)
Carcinoma	2 (4%)			2 (4%)
Bilateral, adenoma	1 (2%)		1 (2%)	
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Urinary bladder	(49)	(49)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		3 (6%)		1 (2%)
Lymphoma, malignant	6 (12%)	5 (10%)	1 (2%)	10 (20%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	26	28	17	25
Total primary neoplasms	37	33	24	34
Total animals with benign neoplasms	17	17	13	14
Total benign neoplasms	19	19	18	17
Total animals with malignant neoplasms	13	13	6	16
Total malignant neoplasms	18	14	6	17
Total animals with metastatic neoplasms	2		1	
Total metastatic neoplasms	2		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 Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Harderian Gland: Adenoma				
Overall rate[a]	5/50 (10%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate[b]	10.4%	2.1%	4.4%	6.4%
Terminal rate[c]	4/45 (9%)	1/44 (2%)	2/36 (6%)	3/43 (7%)
First incidence (days)	621	729 (T)	729 (T)	729 (T)
Poly-3 test[d]	P=0.536N	P=0.105N	P=0.243N	P=0.373N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	1/50 (2%)	2/50 (4%)	5/50 (10%)
Adjusted rate	14.5%	2.1%	4.4%	10.7%
Terminal rate	6/45 (13%)	1/44 (2%)	2/36 (6%)	5/43 (12%)
First incidence (days)	621	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.500	P=0.032N	P=0.095N	P=0.400N
Liver: Hepatocellular Adenoma				
Overall rate	3/50 (6%)	6/50 (12%)	7/49 (14%)	7/50 (14%)
Adjusted rate	6.3%	12.6%	15.6%	14.9%
Terminal rate	3/45 (7%)	6/44 (14%)	6/46 (17%)	7/43 (16%)
First incidence (days)	729 (T)	729 (T)	654	729 (T)
Poly-3 test	P=0.197	P=0.239	P=0.132	P=0.149
Liver: Hepatocellular Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	1/49 (2%)	1/50 (2%)
Adjusted rate	6.3%	6.3%	2.3%	2.1%
Terminal rate	3/45 (7%)	2/44 (5%)	1/36 (3%)	1/43 (2%)
First incidence (days)	729 (T)	719	729 (T)	729 (T)
Poly-3 test	P=0.201N	P=0.659	P=0.332N	P=0.313N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	6/50 (12%)	9/50 (18%)	7/49 (14%)	7/50 (14%)
Adjusted rate	12.5%	18.9%	15.6%	14.9%
Terminal rate	6/45 (13%)	8/44 (18%)	6/36 (17%)	7/43 (16%)
First incidence (days)	729 (T)	719	654	729 (T)
Poly-3 test	P=0.557N	P=0.284	P=0.449	P=0.484
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	2/50 (4%)	5/50 (10%)	1/50 (2%)
Adjusted rate	8.4%	4.2%	10.9%	2.1%
Terminal rate	4/45 (9%)	1/44 (2%)	3/36 (8%)	1/43 (2%)
First incidence (days)	729 (T)	719	633	729 (T)
Poly-3 test	P=0.210N	P=0.340N	P=0.475	P=0.185N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/50 (10%)	3/50 (6%)	5/50 (10%)	2/50 (4%)
Adjusted rate	10.5%	6.3%	10.9%	4.3%
Terminal rate	5/45 (11%)	2/44 (5%)	3/36 (8%)	2/43 (5%)
First incidence (days)	729 (T)	719	633	729 (T)
Poly-3 test	P=0.238N	P=0.360N	P=0.604	P=0.225N
Ovary: Cystadenoma				
Overall rate	3/49 (6%)	2/48 (4%)	1/50 (2%)	0/50 (0%)
Adjusted rate	6.4%	4.3%	2.2%	0.0%
Terminal rate	3/44 (7%)	1/43 (2%)	1/36 (3%)	0/43 (0%)
First incidence (days)	729 (T)	522	729 (T)	—[e]
Poly-3 test	P=0.081N	P=0.499N	P=0.317N	P=0.119N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.2%	2.1%	2.2%	2.1%
Terminal rate	1/45 (2%)	0/44 (0%)	0/36 (0%)	0/43 (0%)
First incidence (days)	535	696	719	521
Poly-3 test	P=0.328N	P=0.312N	P=0.330N	P=0.313N
All Organs: Histiocytic Sarcoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	6.3%	0.0%	2.1%
Terminal rate	0/45 (0%)	2/44 (5%)	0/36 (0%)	0/43 (0%)
First incidence (days)	—	719	—	563
Poly-3 test	P=0.606N	P=0.118	—[f]	P=0.498
All Organs: Malignant Lymphoma				
Overall rate	6/50 (12%)	5/50 (10%)	1/50 (2%)	10/50 (20%)
Adjusted rate	12.4%	10.4%	2.2%	20.9%
Terminal rate	5/45 (11%)	4/44 (9%)	0/36 (0%)	7/43 (16%)
First incidence (days)	516	482	672	544
Poly-3 test	P=0.066	P=0.503N	P=0.067N	P=0.197
All Organs: Benign Neoplasms				
Overall rate	17/50 (34%)	17/50 (34%)	13/50 (26%)	14/50 (28%)
Adjusted rate	34.5%	35.3%	28.0%	29.9%
Terminal rate	14/45 (31%)	15/44 (34%)	9/36 (25%)	14/43 (33%)
First incidence (days)	535	522	633	729 (T)
Poly-3 test	P=0.322N	P=0.553	P=0.321N	P=0.395N
All Organs: Malignant Neoplasms				
Overall rate	13/50 (26%)	13/50 (26%)	6/50 (12%)	16/50 (32%)
Adjusted rate	26.4%	26.8%	13.1%	32.7%
Terminal rate	10/45 (22%)	9/44 (21%)	2/36 (6%)	11/43 (26%)
First incidence (days)	516	482	672	521
Poly-3 test	P=0.240	P=0.575	P=0.085N	P=0.324
All Organs: Benign or Malignant Neoplasms				
Overall rate	26/50 (52%)	28/50 (56%)	17/50 (34%)	25/50 (50%)
Adjusted rate	52.0%	56.9%	36.4%	51.1%
Terminal rate	21/45 (47%)	23/44 (52%)	10/36 (28%)	20/43 (47%)
First incidence (days)	516	482	633	521
Poly-3 test	P=0.423N	P=0.387	P=0.089N	P=0.543N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and ovary; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of the statistic cannot be computed

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	2	2	3
Natural deaths	1	4	12	4
Survivors				
Died last week of study	1			
Terminal sacrifice	44	44	36	43
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(48)	(46)	(39)	(47)
Inflammation, chronic		1 (2%)		
Intestine large, cecum	(48)	(46)	(41)	(47)
Cyst				1 (2%)
Intestine large, rectum	(48)	(47)	(43)	(47)
Intestine small, ileum	(48)	(46)	(39)	(47)
Hyperplasia, lymphoid	1 (2%)			
Intestine small, jejunum	(48)	(46)	(43)	(48)
Hyperplasia, lymphoid		1 (2%)	2 (5%)	
Inflammation, suppurative			1 (2%)	
Liver	(50)	(50)	(49)	(50)
Angiectasis				1 (2%)
Basophilic focus	1 (2%)		3 (6%)	1 (2%)
Clear cell focus		2 (4%)		2 (4%)
Cyst			1 (2%)	2 (4%)
Eosinophilic focus	3 (6%)	3 (6%)	2 (4%)	4 (8%)
Hematopoietic cell proliferation	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Hepatodiaphragmatic nodule				1 (2%)
Infarct				1 (2%)
Infiltration cellular, lymphocyte	24 (48%)	24 (48%)	26 (53%)	21 (42%)
Infiltration cellular, mixed cell	7 (14%)	9 (18%)	10 (20%)	6 (12%)
Inflammation, acute				1 (2%)
Mixed cell focus		3 (6%)	2 (4%)	4 (8%)
Necrosis, focal	1 (2%)	1 (2%)		
Tension lipidosis	3 (6%)	1 (2%)		
Capsule, fibrosis			1 (2%)	
Hepatocyte, vacuolization cytoplasmic	1 (2%)	3 (6%)	7 (14%)	4 (8%)
Kupffer cell, pigmentation				1 (2%)
Mesentery	(10)	(6)	(9)	(2)
Hemorrhage	1 (10%)		1 (11%)	
Infiltration cellular, lymphocyte			1 (11%)	
Fat, necrosis	8 (80%)	6 (100%)	7 (78%)	2 (100%)
Pancreas	(48)	(48)	(47)	(50)
Atrophy	1 (2%)	1 (2%)		1 (2%)
Cyst	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocyte				2 (4%)
Inflammation, granulomatous, chronic		1 (2%)		
Acinus, cytoplasmic alteration	4 (8%)	2 (4%)	1 (2%)	1 (2%)
Artery, inflammation			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 Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Alimentary System (continued)				
Salivary glands	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)	1 (2%)	1 (2%)	
Infiltration cellular, lymphocyte	2 (4%)	2 (4%)	5 (10%)	3 (6%)
Stomach, forestomach	(49)	(50)	(48)	(50)
Cyst			2 (4%)	
Edema		1 (2%)		
Infiltration cellular, lymphocyte			1 (2%)	1 (2%)
Inflammation, granulomatous, chronic		1 (2%)		
Inflammation, chronic	1 (2%)			
Ulcer		1 (2%)	2 (4%)	2 (4%)
Epithelium, hyperplasia		3 (6%)	3 (6%)	2 (4%)
Stomach, glandular	(49)	(49)	(48)	(50)
Cyst	7 (14%)	4 (8%)	2 (4%)	6 (12%)
Ulcer			1 (2%)	
Glands, ectasia				1 (2%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	39 (78%)	38 (76%)	37 (74%)	33 (66%)
Mineralization	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(48)	(50)
Accessory adrenal cortical nodule	1 (2%)			
Angiectasis		1 (2%)	1 (2%)	
Degeneration, cystic	1 (2%)		2 (4%)	1 (2%)
Degeneration, fatty		1 (2%)		
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia, focal	1 (2%)	1 (2%)		
Hypertrophy, focal	1 (2%)	1 (2%)		4 (8%)
Inflammation, granulomatous, chronic		1 (2%)		
Vacuolization cytoplasmic				1 (2%)
Capsule, hyperplasia	49 (98%)	50 (100%)	47 (98%)	49 (98%)
Adrenal medulla	(50)	(50)	(49)	(50)
Amyloid deposition			1 (2%)	
Hyperplasia	1 (2%)	2 (4%)	2 (4%)	
Islets, pancreatic	(48)	(49)	(48)	(50)
Hyperplasia	4 (8%)	2 (4%)	1 (2%)	1 (2%)
Parathyroid gland	(45)	(44)	(48)	(47)
Cyst		1 (2%)		1 (2%)
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)		
Pituitary gland	(50)	(49)	(49)	(49)
Angiectasis	1 (2%)	1 (2%)	1 (2%)	
Pars distalis, cyst			2 (4%)	3 (6%)
Pars distalis, hyperplasia, focal	8 (16%)	5 (10%)	6 (12%)	4 (8%)
Thyroid gland	(50)	(50)	(48)	(50)
Inflammation, suppurative		1 (2%)		
Follicle, cyst		3 (6%)	2 (4%)	2 (4%)
Follicle, degeneration, focal	18 (36%)	18 (36%)	16 (33%)	10 (20%)
Follicular cell, hyperplasia			1 (2%)	1 (2%)
General Body System				
None				

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Genital System				
Clitoral gland	(50)	(50)	(49)	(50)
Cyst	2 (4%)	3 (6%)	4 (8%)	
Ovary	(49)	(48)	(50)	(50)
Angiectasis		1 (2%)		
Cyst	10 (20%)	14 (29%)	10 (20%)	12 (24%)
Hemorrhage	10 (20%)	6 (13%)	7 (14%)	5 (10%)
Mineralization			1 (2%)	
Pigmentation			1 (2%)	
Thrombosis		1 (2%)	1 (2%)	
Bilateral, cyst		1 (2%)		1 (2%)
Corpus luteum, hyperplasia	4 (8%)	1 (2%)		2 (4%)
Granulosa cell, hyperplasia				2 (4%)
Uterus	(50)	(49)	(50)	(50)
Fibrosis				1 (2%)
Hyperplasia, cystic	48 (96%)	46 (94%)	45 (90%)	44 (88%)
Infiltration cellular, histiocyte		1 (2%)		
Inflammation, suppurative	1 (2%)			1 (2%)
Inflammation, granulomatous, chronic		1 (2%)		
Thrombosis		2 (4%)	1 (2%)	
Endometrium, hyperplasia, cystic				
Myometrium, hypertrophy				1 (2%)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Depletion cellular				1 (2%)
Hyperplasia	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Inflammation, chronic			1 (2%)	
Myelofibrosis	6 (12%)	1 (2%)	5 (10%)	5 (10%)
Lymph node	(6)	(4)	(5)	(4)
Iliac, hyperplasia, lymphoid			1 (20%)	
Pancreatic, hyperplasia, lymphoid	1 (17%)		1 (20%)	
Renal, hyperplasia, lymphoid	1 (17%)		1 (20%)	
Lymph node, mandibular	(50)	(49)	(48)	(49)
Hyperplasia, lymphoid	6 (12%)	2 (4%)	4 (8%)	7 (14%)
Pigmentation	1 (2%)			
Lymph node, mesenteric	(48)	(43)	(48)	(49)
Ectasia		3 (7%)	3 (6%)	2 (4%)
Hyperplasia, histiocytic	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid	4 (8%)	1 (2%)	3 (6%)	4 (8%)
Inflammation, granulatomous, chronic		1 (2%)		
Spleen	(48)	(48)	(47)	(49)
Atrophy			1 (2%)	
Hematopoietic cell proliferation	5 (10%)	7 (15%)	8 (17%)	4 (8%)
Lymphoid follicle, hyperplasia	24 (50%)	26 (54%)	18 (38%)	22 (45%)
Thymus	(49)	(48)	(50)	(48)
Atrophy		1 (2%)	1 (2%)	
Hemorrhage	1 (2%)			
Hyperplasia, lymphoid	4 (8%)	2 (4%)	4 (8%)	7 (15%)
Necrosis, lymphoid		1 (2%)		
Integumentary System				
Mammary gland	(49)	(49)	(48)	(50)
Cyst		1 (2%)		
Hyperplasia		1 (2%)		

Table D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Edema	1 (2%)			
Mineralization				1 (2%)
Pigmentation				1 (2%)
Subcutaneous tissue, infiltration cellular, lymphocyte				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fracture			1 (2%)	
Maxilla, fibrosis				1 (2%)
Skeletal muscle	(1)	(1)	(2)	
Necrosis			1 (50%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression		1 (2%)		
Hemorrhage			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body			1 (2%)	
Hemorrhage	2 (4%)		2 (4%)	1 (2%)
Hyperplasia, lymphoid				1 (2%)
Infiltration cellular, histiocyte				1 (2%)
Infiltration cellular, lymphocyte	7 (14%)	2 (4%)	1 (2%)	2 (4%)
Inflammation, suppurative			1 (2%)	
Inflammation, chronic	1 (2%)			
Metaplasia, osseous	1 (2%)			
Alveolar epithelium, hyperplasia	1 (2%)	3 (6%)	2 (4%)	
Nose	(50)	(50)	(50)	(50)
Foreign body			1 (2%)	
Inflammation, suppurative			1 (2%)	
Glands, degeneration, cystic			2 (4%)	1 (2%)
Glands, inflammation				1 (2%)
Respiratory epithelium, hyperplasia				1 (2%)
Trachea	(50)	(50)	(49)	(50)
Glands, degeneration, cystic				1 (2%)
Special Senses System				
Eye	(49)	(47)	(41)	(46)
Atrophy	1 (2%)			
Cornea, inflammation, suppurative	1 (2%)			
Harderian gland	(50)	(49)	(50)	(50)
Hyperplasia			1 (2%)	
Hyperplasia, focal	5 (10%)	2 (4%)	4 (8%)	2 (4%)
Inflammation, chronic	1 (2%)			

Table D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm	3,000 ppm	9,000 ppm	25,000 ppm
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Atrophy		1 (2%)		
Casts protein	2 (4%)		1 (2%)	
Cyst	1 (2%)			
Hydronephrosis		1 (2%)		
Infarct		1 (2%)	3 (6%)	
Infiltration cellular, lymphocyte	7 (14%)	7 (14%)	4 (8%)	9 (18%)
Metaplasia, osseous	1 (2%)	1 (2%)	1 (2%)	
Nephropathy	13 (26%)	19 (39%)	15 (30%)	15 (30%)
Renal tubule, accumulation, hyaline droplet				2 (4%)
Renal tubule, dilatation		1 (2%)		
Transitional epithelium, hyperplasia		1 (2%)		
Urinary bladder	(49)	(49)	(50)	(50)
Infiltration cellular, lymphocyte	7 (14%)	6 (12%)	5 (10%)	3 (6%)

APPENDIX E
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was generally performed as reported by Zeiger *et al.* (1992). Goldenseal root powder and berberine chloride were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). Goldenseal root powder was incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 and *Escherichia coli* strain WP2 *uvrA* pKM101. Berberine chloride was incubated with *S. typhimurium* stains TA97, TA98, TA100, and TA1535. The chemicals were incubated either in buffer or S9 mix [metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster (berberine chloride only) liver] for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of the test chemical. No toxicity was observed for goldenseal root powder and the high dose was limited to 10,000 µg/plate by experimental design. The high dose of berberine chloride was limited by toxicity. All trials were repeated; the second goldenseal root powder *E. coli* trial with 10% rat S9 was invalid due to contamination.

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 end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic (mature) erythrocytes (NCEs) in each of five animals per treatment group. In addition, the percentage of polychromatic (immature) erythrocytes (PCEs) in a population of 1,000 erythrocytes per animal 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 exposure groups with a one-tailed Cochran Armitage trend test, followed by pairwise comparisons between each exposed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month study were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Mouse Bone Marrow Micronucleus Test Protocol

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by berberine chloride administration. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male B6C3F1 mice were injected intraperitoneally [three times at 24-hour intervals] with berberine chloride dissolved in phosphate-buffered saline. Solvent control animals received phosphate-buffered saline only. The positive control animals received injections of cyclophosphamide. The animals were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 PCEs were scored for the frequency of micronucleated cells in each of five animals per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the bone marrow was scored for each dose group as a measure of toxicity.

The PCE results were tabulated as described for NCEs in the mouse peripheral blood micronucleus test.

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

Goldenseal root powder (1,000 to 10,000 µg/plate) was not mutagenic in *S. typhimurium* strains TA100 or TA98 or *E. coli* strain WP2 *uvrA* pKM101 with or without rat liver S9 metabolic activation enzymes (Table E1). No increase in the frequency of micronucleated NCEs was observed in peripheral blood samples from male or female B6C3F1 mice exposed to goldenseal root powder in feed (3,121 to 50,000 ppm) for 3 months; no significant exposure-related changes in the percentages of PCEs were observed in peripheral blood of these mice, suggesting that no exposure-related bone marrow toxicity occurred (Table E2).

Berberine chloride was also shown to be nongenotoxic. Over a concentration range of 0.33 to 1,000 µg/plate, it was not mutagenic in *S. typhimurium* TA97, TA98, TA100, or TA1535 with or without rat or hamster liver S9 metabolic activation enzymes (Table E3). No increases in the frequency of micronucleated PCEs were observed in bone marrow samples from male B6C3F1 mice treated with berberine chloride (41.125 to 658 mg/kg) by intraperitoneal injection 3 times at 24 hour intervals; no dose-related change in the percentage of PCEs in the bone marrow was observed, suggesting that exposure to berberine chloride did not induce bone marrow toxicity (Table E4).

TABLE E1
Mutagenicity of Goldenseal Root Powder in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+ 10% rat S9 Trial 1	+ 10% rat S9 Trial 2
TA100	0	86 ± 3	64 ± 5	77 ± 5	75 ± 5
	1,000	114 ± 2	83 ± 4	87 ± 11	87 ± 2
	1,500			81 ± 3	
	2,500	116 ± 6	86 ± 6		93 ± 3
	5,000	116 ± 10	61 ± 2	78 ± 2	99 ± 4
	7,500	120 ± 2	67 ± 2	77 ± 2	117 ± 6
	10,000	128 ± 10	59 ± 5	69 ± 6	128 ± 11
Trial summary		Negative	Negative	Negative	Negative
Positive control[c]		525 ± 20	650 ± 9	731 ± 11	635 ± 40
TA98	0	29 ± 1	26 ± 4	31 ± 2	29 ± 5
	1,000	48 ± 3	30 ± 2	47 ± 2	36 ± 3
	2,500	37 ± 4	24 ± 1	51 ± 5	45 ± 2
	5,000	23 ± 1	21 ± 4	52 ± 2	37 ± 4
	7,500	17 ± 1	19 ± 4	48 ± 3	36 ± 5
	10,000	11 ± 2	19 ± 4	36 ± 4	29 ± 5
Trial summary		Negative	Negative	Negative	Negative
Positive control		446 ± 55	594 ± 29	896 ± 34	1,188 ± 77
***Escherichia coli* WP2 *uvrA* pKM101 (Analogous to TA102)**					
	0	194 ± 9	174 ± 4	235 ± 7	
	1,000	167 ± 8	236 ± 8	202 ± 19	
	2,500	191 ± 11	244 ± 13	199 ± 4	
	5,000	199 ± 8	279 ± 10	218 ± 2	
	7,500	227 ± 12	258 ± 20	235 ± 3	
	10,000	239 ± 10	262 ± 9	229 ± 11	
Trial summary		Negative	Negative	Negative	
Positive control		2,158 ± 16	1,128 ± 61	1,248 ± 23	

[a] Study was performed at SITEK Research Laboratories. The protocol is a modification of that presented by Zeiger *et al*. (1992); 0 µg/plate was the solvent control
[b] Revertants are presented as mean ± standard error from three plates
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methylmethanesulfonate (pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Goldenseal Root Powder in Feed for 3 Months[a]

Compound	Exposure Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
NTP-2000 feed[d]	0	5	2.1 ± 0.33		3.54 ± 0.25
Goldenseal root powder	3,121	5	1.9 ± 0.43	0.6242	3.74 ± 0.33
	6,250	5	2.7 ± 0.44	0.1930	3.74 ± 0.49
	12,500	5	2.9 ± 0.60	0.1287	4.16 ± 0.47
	25,000	5	2.2 ± 0.20	0.4393	2.96 ± 0.59
	50,000	5	3.1 ± 0.94	0.0825	3.10 ± 0.17
			P=0.087[e]		
Female					
NTP-2000 feed	0	5	1.7 ± 0.46		2.88 ± 0.40
Goldenseal root powder	3,121	5	2.5 ± 0.32	0.1083	2.82 ± 0.28
	6,250	5	1.3 ± 0.37	0.7676	3.50 ± 0.35
	12,500	5	2.4 ± 0.90	0.1369	3.76 ± 0.46
	25,000	5	2.9 ± 0.70	0.0383	3.12 ± 0.54
	50,000	5	1.9 ± 0.37	0.3693	3.66 ± 0.24
			P=0.332		

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

TABLE E3
Mutagenicity of Berberine Chloride in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA100	0	86 ± 2	178 ± 7	167 ± 9	108 ± 4	149 ± 3	128 ± 8
	0.33	93 ± 9	148 ± 5				
	1	107 ± 14	139 ± 3				
	3.3	97 ± 3	145 ± 15	153 ± 13	94 ± 5	158 ± 13	137 ± 3
	10	91 ± 8	140 ± 3	156 ± 14	120 ± 7	159 ± 13	111 ± 14
	33	88 ± 8[c]	134 ± 15	154 ± 6	131 ± 5	173 ± 15	141 ± 4
	100	63 ± 2[c]	47 ± 3[c]	184 ± 10	115 ± 5	150 ± 6	153 ± 4
	333			143 ± 5	116 ± 8[c]	95 ± 6[c]	104 ± 16[c]
	1,000			79 ± 4[c]	71 ± 8	83 ± 9[c]	76 ± 5[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		681 ± 73	359 ± 16	340 ± 16	673 ± 35	660 ± 58	796 ± 40
TA1535	0	8 ± 1	9 ± 2	5 ± 2	10 ± 1	8 ± 1	10 ± 1
	0.33	9 ± 1					
	1	8 ± 1	9 ± 2				
	3.3	6 ± 1	18 ± 2	8 ± 2	11 ± 3	4 ± 1	6 ± 3
	10	7 ± 1	9 ± 3	8 ± 0	9 ± 1	7 ± 2	10 ± 2
	20		15 ± 4				
	33	8 ± 0	20 ± 1	5 ± 1	10 ± 1	5 ± 2	9 ± 2
	50		18 ± 3				
	100	7 ± 1[c]	10 ± 1[c]	2 ± 1	9 ± 2	7 ± 3	7 ± 1
	333			6 ± 2	11 ± 1	5 ± 1[c]	8 ± 1
	1,000			3 ± 1[c]	2 ± 2[c]	1 ± 1[c]	4 ± 1[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		253 ± 21	153 ± 4	24 ± 3	44 ± 5	474 ± 84	107 ± 11

TABLE E3
Mutagenicity of Berberine Chloride in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+ hamster S9 10%	+ hamster S9 30%	+ rat S9 10%	+ rat S9 30%
TA97	0	87 ± 9	133 ± 7	152 ± 3	149 ± 4	132 ± 4	149 ± 10
	0.33	84 ± 5	128 ± 15				
	1	83 ± 11	126 ± 8				
	3.3	89 ± 5	118 ± 6	157 ± 4	131 ± 6	120 ± 4	130 ± 2
	10	92 ± 5	108 ± 6	138 ± 10	151 ± 3	137 ± 8	130 ± 5
	33	104 ± 9	94 ± 3	174 ± 5	137 ± 9	146 ± 9	164 ± 6
	100	35 ± 18[c]	83 ± 7[c]	144 ± 17	193 ± 15	148 ± 12	195 ± 9
	333			118 ± 11[c]	81 ± 4[c]	95 ± 11	62 ± 7[c]
	1,000			81 ± 15[c]	24 ± 6[c]	4 ± 4[c]	6 ± 1[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		297 ± 45	398 ± 8	458 ± 12	716 ± 66	1,334 ± 183	763 ± 61

Strain	Dose (µg/plate)	– S9 Trial 1	– S9 Trial 2	– S9 Trial 3
TA98	0	13 ± 1	12 ± 0	12 ± 2
	0.33	11 ± 1	13 ± 3	
	1	10 ± 1	11 ± 1	17 ± 0
	3.3	10 ± 0	14 ± 2	8 ± 1
	10	12 ± 1	15 ± 2	11 ± 1
	20			7 ± 2
	33	12 ± 1[c]	28 ± 5	10 ± 3
	50			7 ± 1
	100	6 ± 4[c]	6 ± 0[c]	6 ± 1[c]
Trial summary		Negative	Equivocal	Negative
Positive control		154 ± 3	83 ± 7	57 ± 3

Strain	Dose (µg/plate)	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA98	**0**	**17 ± 2**	**17 ± 0**	**15 ± 1**	**17 ± 1**
(continued)	3.3	13 ± 1	22 ± 1	14 ± 1	17 ± 4
	10	17 ± 1	16 ± 0	14 ± 1	14 ± 1
	33	13 ± 0	16 ± 1	10 ± 1	18 ± 2
	100	15 ± 1	17 ± 1	22 ± 3	20 ± 1[c]
	333	11 ± 2	12 ± 2[c]	10 ± 1[c]	8 ± 1[c]
	1,000	4 ± 1[c]	4 ± 0[c]	0 ± 0[c]	6 ± 2[c]
Trial summary		Negative	Negative	Negative	Negative
Positive control		135 ± 7	314 ± 49	101 ± 22	220 ± 32

[a] Study was performed at BioReliance Corporation. The detailed protocol is presented by Zeiger *et al.* (1992). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97) and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E4
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with Berberine Chloride by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	PCEs[b] (%)
Phosphate-buffered saline[d]	0	5	1.5 ± 0.27		68.1 ± 4.07
Berberine chloride	41.125	5	1.8 ± 0.20	0.3006	52.3 ± 2.69
	82.25	5	1.2 ± 0.25	0.7183	63.6 ± 3.77
	164.50	5	1.2 ± 0.25	0.7183	66.0 ± 3.37
	329	5	1.4 ± 0.29	0.5737	65.6 ± 4.84
	658	5	0.8 ± 0.30	0.9279	58.5 ± 4.80
			P=0.946[e]		
Cyclophosphamide[f]	20	5	29.11 ± 4.97	0.0000	54.9 ± 5.45

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by Shelby *et al*. (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the solvent control group; dosed group values are significant at $P \leq 0.005$; positive control values are significant at $P \leq 0.05$ (ILS, 1990)
[d] Solvent control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at $P \leq 0.025$ (ILS, 1990)
[f] Positive control

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1 Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Goldenseal Root Powder 142
TABLE F2 Hematology Data for Mice in the 3-Month Feed Study of Goldenseal Root Powder 147

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
Hematology						
n						
Day 5	9	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 14	5	10	10	10	10	10
Hematocrit (auto) (%)						
Day 5	42.3 ± 0.7	42.1 ± 0.7	43.3 ± 1.2	42.8 ± 0.9	43.7 ± 0.5	44.9 ± 0.7*
Day 23	47.3 ± 0.7	46.4 ± 0.5	47.1 ± 0.6	47.1 ± 0.7	47.1 ± 0.7	47.5 ± 1.6
Week 14	46.0 ± 0.7	45.5 ± 0.4	46.2 ± 0.4	45.4 ± 0.3	45.2 ± 0.5	44.9 ± 0.4
Hematocrit (spun) (%)						
Day 5	41.5 ± 0.5[b]	41.0 ± 0.6	42.2 ± 1.1	41.6 ± 0.8	42.5 ± 0.4	43.8 ± 0.5*
Day 23	47.1 ± 0.6	46.4 ± 0.3	46.4 ± 0.6	46.7 ± 0.6	46.8 ± 0.6	47.0 ± 1.2
Week 14	46.5 ± 0.6	45.9 ± 0.5	46.1 ± 0.4	45.5 ± 0.4	45.5 ± 0.3	44.9 ± 0.3*
Hemoglobin (g/dL)						
Day 5	13.8 ± 0.2	13.7 ± 0.2	14.1 ± 0.4	14.0 ± 0.3	14.3 ± 0.1	14.7 ± 0.2**
Day 23	15.8 ± 0.2	15.6 ± 0.2	15.6 ± 0.2	15.7 ± 0.2	15.8 ± 0.2	15.8 ± 0.5
Week 14	15.2 ± 0.2	14.9 ± 0.1	14.9 ± 0.1	14.7 ± 0.1	14.6 ± 0.1*	14.5 ± 0.1*
Erythrocytes (10^6/µL)						
Day 5	7.12 ± 0.13	7.08 ± 0.11	7.21 ± 0.19	7.18 ± 0.15	7.41 ± 0.08	7.65 ± 0.13*
Day 23	8.05 ± 0.12	7.86 ± 0.09	7.91 ± 0.11	8.05 ± 0.12	8.15 ± 0.13	8.27 ± 0.31
Week 14	9.21 ± 0.14	9.00 ± 0.05	9.04 ± 0.06	9.00 ± 0.07	9.00 ± 0.08	9.11 ± 0.09
Reticulocytes (10^5/µL)						
Day 5	5.9 ± 0.2	5.7 ± 0.1	6.0 ± 0.2	5.7 ± 0.2	5.3 ± 0.1*	4.4 ± 0.1**
Day 23	2.9 ± 0.2	2.9 ± 0.2	3.3 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.3 ± 0.3
Week 14	1.9 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.0 ± 0.0	2.0 ± 0.1
Nucleated erythrocytes (10^3/µL)						
Day 5	0.4 ± 0.2[b]	0.5 ± 0.3	1.1 ± 0.7	0.7 ± 0.3	1.0 ± 0.3	0.9 ± 0.2
Day 23	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0
Week 14	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1
Mean cell volume (fL)						
Day 5	59.4 ± 0.5	59.4 ± 0.2	60.1 ± 0.3	59.6 ± 0.3	59.1 ± 0.4	58.7 ± 0.4
Day 23	58.7 ± 0.3	59.1 ± 0.2	59.5 ± 0.2	58.5 ± 0.3	57.9 ± 0.3	57.5 ± 0.4*
Week 14	49.9 ± 0.1	50.6 ± 0.2	51.0 ± 0.2	50.4 ± 0.2	50.3 ± 0.2	49.2 ± 0.1
Mean cell hemoglobin (pg)						
Day 5	19.4 ± 0.1	19.4 ± 0.1	19.6 ± 0.1	19.5 ± 0.1	19.3 ± 0.1	19.2 ± 0.1
Day 23	19.6 ± 0.1	19.8 ± 0.1	19.8 ± 0.1	19.5 ± 0.1	19.3 ± 0.1	19.1 ± 0.2*
Week 14	16.5 ± 0.1	16.5 ± 0.1	16.5 ± 0.1	16.3 ± 0.0	16.2 ± 0.1*	16.0 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 5	32.7 ± 0.2	32.6 ± 0.1	32.6 ± 0.1	32.7 ± 0.2	32.6 ± 0.3	32.8 ± 0.2
Day 23	33.4 ± 0.1	33.6 ± 0.1	33.2 ± 0.1	33.3 ± 0.2	33.5 ± 0.1	33.2 ± 0.2
Week 14	33.0 ± 0.1	32.6 ± 0.1	32.3 ± 0.2	32.3 ± 0.1*	32.3 ± 0.2*	32.4 ± 0.1
Platelets (10^3/µL)						
Day 5	862.7 ± 35.3	799.8 ± 17.2	759.7 ± 24.6	876.0 ± 34.6	907.9 ± 40.8	942.7 ± 31.6
Day 23	854.4 ± 27.1	796.1 ± 22.3	856.4 ± 24.4	808.6 ± 19.9	814.7 ± 28.4	866.8 ± 46.0
Week 14	667.6 ± 12.1	637.3 ± 22.3	664.5 ± 7.9	722.2 ± 20.7	722.9 ± 13.4	740.3 ± 18.1*
Leukocytes (10^3/µL)						
Day 5	8.71 ± 0.49	9.17 ± 0.43	8.76 ± 0.43	9.52 ± 0.38	10.33 ± 0.44*	10.16 ± 0.31*
Day 23	11.02 ± 0.20	10.95 ± 0.23	11.11 ± 0.44	11.79 ± 0.34	11.40 ± 0.52	11.15 ± 0.31
Week 14	9.99 ± 0.37	9.69 ± 0.16	10.03 ± 0.32	10.39 ± 0.27	9.74 ± 0.22	9.59 ± 0.24
Segmented neutrophils (10^3/µL)						
Day 5	0.89 ± 0.04	1.02 ± 0.07	0.93 ± 0.06	1.06 ± 0.04*	1.18 ± 0.05**	1.11 ± 0.03**
Day 23	0.94 ± 0.03	0.94 ± 0.04	1.02 ± 0.05	1.16 ± 0.08	1.18 ± 0.07	0.87 ± 0.02
Week 14	1.12 ± 0.07	1.13 ± 0.06	1.29 ± 0.11	1.23 ± 0.07	0.98 ± 0.02	1.11 ± 0.06

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Goldenseal Root Powder

	0 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male (continued)						
Hematology (continued)						
n						
Day 5	9	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 14	5	10	10	10	10	10
Lymphocytes (10^3/µL)						
Day 5	7.51 ± 0.44	7.79 ± 0.34	7.51 ± 0.37	8.09 ± 0.34	8.79 ± 0.38	8.67 ± 0.27
Day 23	9.75 ± 0.19	9.68 ± 0.19	9.77 ± 0.42	10.25 ± 0.30	9.87 ± 0.45	9.95 ± 0.30
Week 14	8.52 ± 0.39	8.23 ± 0.12	8.38 ± 0.35	8.80 ± 0.25	8.44 ± 0.22	8.11 ± 0.20
Monocytes (10^3/µL)						
Day 5	0.15 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.20 ± 0.02
Day 23	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.13 ± 0.02
Week 14	0.17 ± 0.01	0.16 ± 0.01	0.19 ± 0.02	0.18 ± 0.01	0.15 ± 0.01	0.19 ± 0.01
Basophils (10^3/µL)						
Day 5	0.066 ± 0.014	0.065 ± 0.007	0.063 ± 0.008	0.071 ± 0.008	0.074 ± 0.012	0.066 ± 0.008
Day 23	0.058 ± 0.006	0.048 ± 0.002	0.052 ± 0.007	0.069 ± 0.009	0.058 ± 0.004	0.056 ± 0.005
Week 14	0.038 ± 0.005	0.036 ± 0.003	0.035 ± 0.004	0.040 ± 0.003	0.037 ± 0.003	0.038 ± 0.003
Eosinophils (10^3/µL)						
Day 5	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.00
Day 23	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.00
Week 14	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.06 ± 0.00**	0.05 ± 0.00**
Large unstained cells (10^3/µL)						
Day 5	0.062 ± 0.007	0.091 ± 0.011	0.073 ± 0.010	0.086 ± 0.008	0.085 ± 0.010	0.098 ± 0.010
Day 23	0.104 ± 0.009	0.106 ± 0.008	0.099 ± 0.011	0.120 ± 0.012	0.117 ± 0.010	0.115 ± 0.011
Week 14	0.072 ± 0.007	0.074 ± 0.003	0.070 ± 0.006	0.079 ± 0.004	0.087 ± 0.008	0.089 ± 0.007
Clinical Chemistry						
n						
Day 5	10	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 14	5	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 5	12.1 ± 0.3	12.0 ± 0.4	11.8 ± 0.4	12.5 ± 0.6	12.1 ± 0.3	13.3 ± 0.3
Day 23	14.7 ± 0.5	15.0 ± 0.5	13.1 ± 0.5	18.8 ± 1.5	21.8 ± 1.9**	17.3 ± 0.8*
Week 14	20.8 ± 0.8	17.6 ± 0.3**	19.8 ± 0.5	19.5 ± 0.6	21.1 ± 0.6	20.4 ± 0.5
Creatinine (mg/dL)						
Day 5	0.45 ± 0.02	0.48 ± 0.01[c]	0.50 ± 0.00	0.48 ± 0.01	0.50 ± 0.00	0.50 ± 0.02
Day 23	0.53 ± 0.02	0.52 ± 0.01	0.58 ± 0.02	0.52 ± 0.01	0.50 ± 0.00	0.55 ± 0.02
Week 14	0.62 ± 0.04	0.65 ± 0.02	0.68 ± 0.02	0.66 ± 0.02	0.66 ± 0.02	0.62 ± 0.02
Total protein (g/dL)						
Day 5	5.4 ± 0.1	5.4 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.9 ± 0.1**
Day 23	6.1 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.5 ± 0.1*	6.6 ± 0.1**
Week 14	6.4 ± 0.2	6.4 ± 0.1	6.6 ± 0.1	6.8 ± 0.1**	6.9 ± 0.1**	7.1 ± 0.1**
Albumin (g/dL)						
Day 5	3.8 ± 0.1	3.7 ± 0.0	3.8 ± 0.0	3.8 ± 0.0	3.8 ± 0.0	4.0 ± 0.0
Day 23	4.3 ± 0.1	4.2 ± 0.0	4.3 ± 0.0	4.4 ± 0.1	4.5 ± 0.1*	4.5 ± 0.1*
Week 14	4.5 ± 0.1	4.5 ± 0.0	4.5 ± 0.0	4.6 ± 0.0	4.7 ± 0.0**	5.0 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 5	55 ± 3	51 ± 2[c]	49 ± 4[c]	48 ± 3	50 ± 3	48 ± 4
Day 23	47 ± 1	43 ± 2	43 ± 2	44 ± 2	43 ± 2	33 ± 1**
Week 14	124 ± 7	71 ± 4**	57 ± 2**	50 ± 2**	50 ± 2**	42 ± 1**

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Goldenseal Root Powder

	0 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 5	10	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 14	5	10	10	10	10	10
Alkaline phosphatase (IU/L)						
Day 5	678 ± 27	652 ± 14	661 ± 17	672 ± 11	651 ± 13	632 ± 18
Day 23	442 ± 15	410 ± 9	435 ± 8	346 ± 19**	293 ± 16**	315 ± 13**
Week 14	220 ± 14	191 ± 4	187 ± 5	184 ± 3*	183 ± 4*	178 ± 3**
Creatine kinase (IU/L)						
Day 5	475 ± 98	405 ± 32	425 ± 54	415 ± 22	384 ± 44	347 ± 39
Day 23	350 ± 36	279 ± 29	314 ± 23	302 ± 22	293 ± 18	286 ± 21
Week 14	252 ± 33	263 ± 30	250 ± 19	270 ± 32	265 ± 40	277 ± 40
Sorbitol dehydrogenase (IU/L)						
Day 5	19 ± 2	19 ± 1[d]	22 ± 1[c]	17 ± 1	22 ± 1	23 ± 1[c]
Day 23	16 ± 1	16 ± 1	16 ± 1	15 ± 1	16 ± 1	17 ± 1
Week 14	25 ± 2	17 ± 1*	18 ± 1*	15 ± 1**	16 ± 1**	15 ± 1**
Bile acids (µmol/L)						
Day 5	42.8 ± 2.2	42.4 ± 2.0[c]	41.9 ± 2.7	38.5 ± 1.6	49.5 ± 1.8	43.9 ± 2.1
Day 23	28.0 ± 1.5	28.3 ± 1.3	30.9 ± 1.4	27.3 ± 1.9	26.3 ± 0.8	33.7 ± 1.6
Week 14	25.4 ± 4.2	21.6 ± 2.3	21.9 ± 2.2	21.7 ± 1.5	29.1 ± 2.7	33.0 ± 1.8
Female						
Hematology						
n						
Day 5	10	10	10	10	10	10
Day 23	9	10	10	8	10	10
Week 14	10	10	10	10	10	10
Hematocrit (auto) (%)						
Day 5	41.3 ± 0.5	41.4 ± 0.5	42.1 ± 0.5	42.4 ± 0.9	43.9 ± 0.6**	43.1 ± 0.5*
Day 23	46.7 ± 0.7	44.7 ± 0.6	46.1 ± 0.6	46.1 ± 0.4	46.6 ± 0.5	45.1 ± 0.5
Week 14	43.6 ± 0.4	43.3 ± 0.3	46.2 ± 2.2	42.9 ± 0.3	43.7 ± 0.3	43.0 ± 0.3
Hematocrit (spun) (%)						
Day 5	41.3 ± 0.4	41.2 ± 0.6	42.5 ± 0.5	42.2 ± 0.8	43.5 ± 0.8*	43.2 ± 0.6*
Day 23	47.9 ± 0.7	45.7 ± 0.6*	47.2 ± 0.5	47.1 ± 0.5	47.6 ± 0.6	46.2 ± 0.5
Week 14	43.9 ± 0.3	43.8 ± 0.5	46.6 ± 2.1	43.5 ± 0.3	44.3 ± 0.3	43.2 ± 0.3
Hemoglobin (g/dL)						
Day 5	13.9 ± 0.2	13.8 ± 0.2	14.1 ± 0.1	14.3 ± 0.3	14.6 ± 0.2*	14.6 ± 0.2*
Day 23	15.6 ± 0.2	15.0 ± 0.2	15.4 ± 0.2	15.2 ± 0.1	15.5 ± 0.2	15.0 ± 0.2
Week 14	14.6 ± 0.1	14.4 ± 0.1	15.2 ± 0.6	14.3 ± 0.1	14.5 ± 0.1	14.2 ± 0.1*
Erythrocytes (10^6/µL)						
Day 5	7.37 ± 0.10	7.32 ± 0.10	7.53 ± 0.08	7.57 ± 0.16	7.85 ± 0.11**	7.74 ± 0.10*
Day 23	8.12 ± 0.14	7.74 ± 0.10	8.04 ± 0.11	7.98 ± 0.08	8.16 ± 0.09	8.02 ± 0.08
Week 14	8.52 ± 0.06	8.44 ± 0.10	8.92 ± 0.32	8.43 ± 0.05	8.59 ± 0.05	8.59 ± 0.05
Reticulocytes (10^5/µL)						
Day 5	4.6 ± 0.2	4.7 ± 0.1	4.5 ± 0.2	4.7 ± 0.3	4.4 ± 0.2	3.7 ± 0.1**
Day 23	2.0 ± 0.1	2.2 ± 0.2	1.8 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1
Week 14	1.8 ± 0.0	2.1 ± 0.5	2.2 ± 0.5	1.9 ± 0.1	1.6 ± 0.1	1.8 ± 0.1

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Goldenseal Root Powder

	0 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Female (continued)						
Hematology (continued)						
n						
Day 5	10	10	10	10	10	10
Day 23	9	10	10	8	10	10
Week 14	10	10	10	10	10	10
Nucleated erythrocytes (10^3/µL)						
Day 5	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1
Day 23	0.2 ± 0.1	0.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
Week 14	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
Mean cell volume (fL)						
Day 5	56.1 ± 0.5	56.5 ± 0.3	55.9 ± 0.5	56.0 ± 0.5	55.9 ± 0.4	55.7 ± 0.3
Day 23	57.5 ± 0.3	57.8 ± 0.3	57.3 ± 0.3	57.8 ± 0.2	57.2 ± 0.3	56.3 ± 0.3*
Week 14	51.2 ± 0.1	51.4 ± 0.3	51.6 ± 0.5	50.9 ± 0.1	50.9 ± 0.3	50.0 ± 0.2**
Mean cell hemoglobin (pg)						
Day 5	18.8 ± 0.1	18.8 ± 0.1	18.8 ± 0.1	18.8 ± 0.1	18.6 ± 0.1	18.9 ± 0.1
Day 23	19.2 ± 0.2	19.3 ± 0.1	19.2 ± 0.1	19.1 ± 0.2	18.9 ± 0.1	18.7 ± 0.1**
Week 14	17.1 ± 0.0	17.0 ± 0.1	17.0 ± 0.1	17.0 ± 0.0*	16.9 ± 0.1**	16.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 5	33.6 ± 0.2	33.3 ± 0.2	33.6 ± 0.3	33.6 ± 0.3	33.3 ± 0.2	34.0 ± 0.2
Day 23	33.4 ± 0.2	33.4 ± 0.2	33.4 ± 0.1	33.0 ± 0.2	33.1 ± 0.1	33.2 ± 0.1
Week 14	33.4 ± 0.1	33.2 ± 0.1	32.9 ± 0.3	33.3 ± 0.1	33.2 ± 0.1	33.1 ± 0.1
Platelets (10^3/µL)						
Day 5	782.8 ± 40.2	795.0 ± 28.2	832.0 ± 25.7	833.8 ± 27.8	818.8 ± 33.2	844.2 ± 30.8
Day 23	726.4 ± 36.1	681.2 ± 30.6	687.4 ± 23.0	774.4 ± 29.3	779.5 ± 23.6	750.3 ± 23.6
Week 14	687.6 ± 24.1	672.0 ± 28.8	610.6 ± 30.6	689.5 ± 25.7	695.8 ± 25.7	742.5 ± 18.8
Leukocytes (10^3/µL)						
Day 5	10.18 ± 0.37	9.56 ± 0.31	10.10 ± 0.38	9.47 ± 0.17	9.56 ± 0.40	10.24 ± 0.57
Day 23	10.25 ± 0.45	10.22 ± 0.59	10.75 ± 0.33	11.53 ± 0.68	11.54 ± 0.32	10.48 ± 0.44
Week 14	7.92 ± 0.42	6.75 ± 0.30	6.97 ± 0.47	6.69 ± 0.22	7.85 ± 0.24	7.69 ± 0.31
Segmented neutrophils (10^3/µL)						
Day 5	0.94 ± 0.06	0.90 ± 0.04	1.12 ± 0.08	0.89 ± 0.02	0.93 ± 0.04	0.93 ± 0.06
Day 23	0.89 ± 0.04	0.94 ± 0.04	0.92 ± 0.06	1.14 ± 0.10	1.03 ± 0.05	0.91 ± 0.07
Week 14	1.15 ± 0.04	0.91 ± 0.07	0.99 ± 0.08	0.89 ± 0.06	1.44 ± 0.08	0.90 ± 0.06
Lymphocytes (10^3/µL)						
Day 5	8.88 ± 0.30	8.30 ± 0.27	8.60 ± 0.30	8.23 ± 0.15	8.29 ± 0.36	8.91 ± 0.49
Day 23	9.02 ± 0.39	8.96 ± 0.54	9.50 ± 0.31	10.04 ± 0.59	10.14 ± 0.30	9.25 ± 0.37
Week 14	6.50 ± 0.41	5.60 ± 0.24	5.75 ± 0.46	5.59 ± 0.21	6.13 ± 0.20	6.52 ± 0.28
Monocytes (10^3/µL)						
Day 5	0.17 ± 0.02	0.18 ± 0.01	0.18 ± 0.02	0.17 ± 0.01	0.18 ± 0.02	0.19 ± 0.02
Day 23	0.14 ± 0.02	0.13 ± 0.02	0.13 ± 0.01	0.14 ± 0.02	0.16 ± 0.01	0.14 ± 0.02
Week 14	0.13 ± 0.02	0.11 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
Basophils (10^3/µL)						
Day 5	0.075 ± 0.010	0.061 ± 0.011	0.071 ± 0.012	0.060 ± 0.008	0.051 ± 0.006	0.068 ± 0.014
Day 23	0.050 ± 0.003	0.055 ± 0.008	0.050 ± 0.003	0.054 ± 0.004	0.058 ± 0.002	0.052 ± 0.005
Week 14	0.027 ± 0.003	0.020 ± 0.004	0.023 ± 0.003	0.022 ± 0.003	0.024 ± 0.003	0.027 ± 0.003
Eosinophils (10^3/µL)						
Day 5	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Day 23	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
Week 14	0.05 ± 0.00	0.06 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.00	0.04 ± 0.00
Large unstained cells (10^3/µL)						
Day 5	0.085 ± 0.012	0.088 ± 0.007	0.099 ± 0.008	0.077 ± 0.008	0.074 ± 0.010	0.109 ± 0.012
Day 23	0.101 ± 0.013	0.102 ± 0.015	0.102 ± 0.009	0.115 ± 0.011	0.116 ± 0.007	0.090 ± 0.009
Week 14	0.060 ± 0.010	0.056 ± 0.005	0.044 ± 0.006	0.044 ± 0.004	0.062 ± 0.009	0.073 ± 0.007

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Feed Study of Goldenseal Root Powder

	0 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Female (continued)						
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 5	10.9 ± 0.5	10.6 ± 0.3	11.0 ± 0.3	12.0 ± 0.7	12.0 ± 0.5	13.5 ± 0.4**
Day 23	16.4 ± 0.6	17.8 ± 0.5	16.9 ± 0.4	15.4 ± 0.5	16.0 ± 0.4	16.1 ± 0.6
Week 14	17.6 ± 0.6	17.3 ± 0.4	18.9 ± 1.2	16.7 ± 0.4	17.0 ± 0.5	19.9 ± 0.5
Creatinine (mg/dL)						
Day 5	0.43 ± 0.02	0.42 ± 0.01	0.44 ± 0.02	0.44 ± 0.02	0.44 ± 0.02	0.45 ± 0.02
Day 23	0.54 ± 0.02	0.51 ± 0.01	0.51 ± 0.01	0.53 ± 0.02	0.50 ± 0.00	0.49 ± 0.01*
Week 14	0.61 ± 0.01	0.62 ± 0.01	0.62 ± 0.01	0.62 ± 0.02	0.59 ± 0.01	0.59 ± 0.01
Total protein (g/dL)						
Day 5	5.5 ± 0.1	5.4 ± 0.1	5.5 ± 0.1	5.7 ± 0.1	5.8 ± 0.1*	6.0 ± 0.1**
Day 23	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.5 ± 0.1*
Week 14	6.3 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.6 ± 0.1*	7.0 ± 0.1**	7.1 ± 0.1**
Albumin (g/dL)						
Day 5	4.0 ± 0.1	3.9 ± 0.0	3.9 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.2 ± 0.0**
Day 23	4.6 ± 0.1	4.4 ± 0.1*	4.4 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.6 ± 0.1
Week 14	4.8 ± 0.1	4.9 ± 0.0	4.8 ± 0.1	4.8 ± 0.1	5.0 ± 0.0**	5.1 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 5	48 ± 2	50 ± 2	47 ± 3	46 ± 3	44 ± 2	46 ± 2
Day 23	36 ± 1	41 ± 2	40 ± 1	36 ± 1	34 ± 1	34 ± 1
Week 14	65 ± 6	43 ± 2**	43 ± 2**	35 ± 1**	39 ± 1**	36 ± 1**
Alkaline phosphatase (IU/L)						
Day 5	539 ± 9	512 ± 14	502 ± 15	510 ± 17	511 ± 16	501 ± 14
Day 23	327 ± 7	298 ± 5**	288 ± 5**	281 ± 9**	268 ± 6**	254 ± 5**
Week 14	176 ± 6	138 ± 4**	141 ± 5**	137 ± 6**	129 ± 4**	124 ± 2**
Creatine kinase (IU/L)						
Day 5	411 ± 26	353 ± 20	345 ± 21	348 ± 29	324 ± 22	362 ± 35
Day 23	320 ± 25	281 ± 14	307 ± 32	295 ± 34	305 ± 21	242 ± 21
Week 14	222 ± 26	209 ± 29	197 ± 19	176 ± 21	155 ± 14	207 ± 30
Sorbitol dehydrogenase (IU/L)						
Day 5	17 ± 1	20 ± 2	19 ± 1	22 ± 1	20 ± 1	20 ± 1
Day 23	20 ± 1	22 ± 1	21 ± 1	21 ± 1	22 ± 1	23 ± 1
Week 14	20 ± 1	16 ± 1*	17 ± 1	16 ± 1*	18 ± 1	18 ± 1
Bile acids (μmol/L)						
Day 5	27.6 ± 2.0	31.0 ± 2.0	27.8 ± 1.6	30.8 ± 1.5	30.6 ± 1.0	37.1 ± 1.7**
Day 23	24.0 ± 1.7	22.7 ± 1.0	27.3 ± 2.9	26.3 ± 1.6	29.6 ± 1.3*	32.6 ± 1.6**
Week 14	30.8 ± 2.8	29.5 ± 3.2	31.4 ± 3.1	34.2 ± 3.5	38.5 ± 2.3	37.0 ± 2.1

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=10
[c] n=9
[d] n=8

TABLE F2
Hematology Data for Mice in the 3-Month Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10	10	10
Male						
Hematocrit (auto) (%)	47.8 ± 0.5	47.6 ± 0.7	47.1 ± 0.8	48.0 ± 0.6	46.9 ± 0.6	49.3 ± 0.5
Hematocrit (%)	47.9 ± 0.5	48.3 ± 0.7	48.0 ± 0.7	48.3 ± 0.7	47.8 ± 0.6	49.8 ± 0.5
Hemoglobin (g/dL)	16.6 ± 0.2	16.3 ± 0.2	16.3 ± 0.3	16.6 ± 0.2	16.3 ± 0.2	17.1 ± 0.2
Erythrocytes (10^6/µL)	11.05 ± 0.13	10.76 ± 0.15	10.65 ± 0.19	10.90 ± 0.14	10.63 ± 0.14	11.20 ± 0.12
Reticulocytes (10^5/µL)	2.7 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.1 ± 0.2*	2.6 ± 0.0
Nucleated erythrocytes (10^3/µL)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)	43.3 ± 0.3	44.3 ± 0.3*	44.3 ± 0.3	44.1 ± 0.1	44.1 ± 0.2	44.0 ± 0.2
Mean cell hemoglobin (pg)	15.0 ± 0.1	15.2 ± 0.1	15.3 ± 0.1*	15.3 ± 0.1*	15.4 ± 0.1**	15.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	34.7 ± 0.1	34.3 ± 0.2	34.5 ± 0.2	34.7 ± 0.1	34.8 ± 0.2	34.8 ± 0.2
Platelets (10^3/µL)	949.0 ± 65.7	945.9 ± 47.4	983.7 ± 67.1	1,050.3 ± 75.5	1,016.1 ± 68.7	958.6 ± 42.9
Leukocytes (10^3/µL)	5.60 ± 0.70	3.16 ± 0.45	3.78 ± 0.81	3.40 ± 0.51	3.81 ± 0.49	3.79 ± 0.49
Segmented neutrophils (10^3/µL)	0.53 ± 0.08	0.37 ± 0.04	0.44 ± 0.10	0.45 ± 0.10	0.44 ± 0.06	0.41 ± 0.10
Lymphocytes (10^3/µL)	4.82 ± 0.60	2.64 ± 0.41*	3.16 ± 0.70	2.81 ± 0.41	3.21 ± 0.43	3.22 ± 0.39
Monocytes (10^3/µL)	0.07 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Basophils (10^3/µL)	0.022 ± 0.003	0.013 ± 0.004	0.014 ± 0.005	0.012 ± 0.003	0.014 ± 0.003	0.017 ± 0.004
Eosinophils (10^3/µL)	0.12 ± 0.02	0.10 ± 0.01	0.11 ± 0.02	0.09 ± 0.02	0.10 ± 0.01	0.10 ± 0.01
Large unstained cells (10^3/µL)	0.028 ± 0.005	0.012 ± 0.003	0.011 ± 0.005	0.009 ± 0.003*	0.012 ± 0.004	0.011 ± 0.003
Female						
Hematocrit (auto) (%)	48.0 ± 0.9	46.6 ± 0.7	48.5 ± 0.7	48.7 ± 0.5	49.2 ± 0.9	49.0 ± 0.6
Hematocrit (spun) (%)	47.8 ± 0.7	47.0 ± 0.6	48.6 ± 0.7	48.6 ± 0.5	49.2 ± 0.7	49.2 ± 0.6
Hemoglobin (g/dL)	16.1 ± 0.3	15.7 ± 0.2	16.3 ± 0.2	16.4 ± 0.2	16.5 ± 0.3	16.6 ± 0.2
Erythrocytes (10^6/µL)	10.73 ± 0.18	10.37 ± 0.15	10.88 ± 0.15	10.95 ± 0.11	11.09 ± 0.20	11.25 ± 0.12*
Reticulocytes (10^5/µL)	2.2 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.3 ± 0.1	2.4 ± 0.1
Nucleated erythrocytes (10^3/µL)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)	44.8 ± 0.1	45.0 ± 0.2	44.6 ± 0.1	44.5 ± 0.2	44.4 ± 0.1	43.5 ± 0.2**
Mean cell hemoglobin (pg)	15.0 ± 0.1	15.1 ± 0.0	15.0 ± 0.1	15.0 ± 0.1	14.9 ± 0.1	14.7 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	33.5 ± 0.1	33.6 ± 0.1	33.6 ± 0.1	33.8 ± 0.1	33.6 ± 0.2	33.9 ± 0.2
Platelets (10^3/µL)	763.2 ± 55.0	896.0 ± 60.5	772.1 ± 57.2	748.8 ± 50.1	798.5 ± 60.2	735.9 ± 38.2
Leukocytes (10^3/µL)	5.28 ± 0.33	5.91 ± 0.36	4.94 ± 0.26	5.68 ± 0.30	5.50 ± 0.32	5.27 ± 0.42
Segmented neutrophils (10^3/µL)	0.66 ± 0.06	0.66 ± 0.06	0.55 ± 0.03	0.62 ± 0.05	0.45 ± 0.04**	0.53 ± 0.07*
Lymphocytes (10^3/µL)	4.28 ± 0.26	4.85 ± 0.32	4.07 ± 0.26	4.72 ± 0.26	4.73 ± 0.28	4.42 ± 0.34
Monocytes (10^3/µL)	0.07 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Basophils (10^3/µL)	0.017 ± 0.003	0.028 ± 0.004	0.023 ± 0.008	0.020 ± 0.001	0.023 ± 0.004	0.020 ± 0.002
Eosinophils (10^3/µL)	0.22 ± 0.02	0.25 ± 0.03	0.21 ± 0.03	0.22 ± 0.03	0.19 ± 0.01	0.19 ± 0.03
Large unstained cells (10^3/µL)	0.028 ± 0.002	0.032 ± 0.004	0.021 ± 0.002	0.025 ± 0.004	0.026 ± 0.004	0.028 ± 0.004

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

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

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TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Feed Study of Goldenseal Root Powder[a]

	0 ppm	1,560 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
n	5	5	5	5	5	5	5
Male							
Necropsy body wt	209 ± 5	212 ± 3	212 ± 4	210 ± 5	210 ± 3	204 ± 6	201 ± 8
Brain							
Absolute	1.783 ± 0.070	1.816 ± 0.022	1.871 ± 0.039	1.817 ± 0.043	1.859 ± 0.022	1.806 ± 0.045	1.826 ± 0.052
Relative	8.513 ± 0.214	8.556 ± 0.060	8.852 ± 0.245	8.650 ± 0.180	8.879 ± 0.164	8.841 ± 0.143	9.122 ± 0.246
Heart							
Absolute	0.71 ± 0.02	0.75 ± 0.01	0.73 ± 0.02	0.75 ± 0.03	0.76 ± 0.02	0.71 ± 0.02	0.72 ± 0.04
Relative	3.379 ± 0.059	3.550 ± 0.017	3.465 ± 0.074	3.577 ± 0.050	3.608 ± 0.088	3.490 ± 0.078	3.595 ± 0.107
R. Kidney							
Absolute	0.89 ± 0.02	0.89 ± 0.02	0.88 ± 0.03	0.93 ± 0.02	0.93 ± 0.03	0.87 ± 0.03	0.88 ± 0.03
Relative	4.248 ± 0.052	4.213 ± 0.060	4.158 ± 0.066	4.432 ± 0.100	4.442 ± 0.101	4.242 ± 0.067	4.375 ± 0.051
Liver							
Absolute	9.63 ± 0.26	10.53 ± 0.26	10.65 ± 0.22	11.76 ± 0.14**	12.74 ± 0.32**	13.50 ± 0.38**	14.29 ± 0.74**
Relative	46.017 ± 0.421	49.568 ± 0.869*	50.296 ± 0.603**	56.021 ± 0.837**	60.816 ± 1.378**	66.048 ± 0.745**	71.048 ± 1.455**
Lung							
Absolute	1.05 ± 0.03	1.18 ± 0.05	1.07 ± 0.05	1.15 ± 0.05	1.14 ± 0.04	1.04 ± 0.07	1.10 ± 0.05
Relative	5.028 ± 0.071	5.547 ± 0.182	5.057 ± 0.197	5.448 ± 0.178	5.450 ± 0.194	5.061 ± 0.234	5.454 ± 0.155
R. Testis							
Absolute	1.180 ± 0.010	1.232 ± 0.025	1.177 ± 0.023	1.195 ± 0.033	1.212 ± 0.031	1.201 ± 0.046	1.181 ± 0.044
Relative	5.651 ± 0.130	5.799 ± 0.060	5.565 ± 0.096	5.691 ± 0.151	5.786 ± 0.122	5.876 ± 0.150	5.908 ± 0.280
Thymus							
Absolute	0.452 ± 0.021	0.479 ± 0.031	0.485 ± 0.034	0.496 ± 0.027	0.465 ± 0.012	0.444 ± 0.031	0.480 ± 0.024
Relative	2.155 ± 0.062	2.253 ± 0.134	2.287 ± 0.140	2.362 ± 0.116	2.221 ± 0.062	2.174 ± 0.137	2.389 ± 0.067
Female							
Necropsy body wt	145 ± 3	141 ± 2	145 ± 3	139 ± 4	142 ± 3	143 ± 2	142 ± 4
Brain							
Absolute	1.746 ± 0.016	1.759 ± 0.025	1.787 ± 0.037	1.718 ± 0.041	1.747 ± 0.044	1.755 ± 0.029	1.758 ± 0.037
Relative	12.059 ± 0.286	12.532 ± 0.298	12.337 ± 0.269	12.342 ± 0.301	12.317 ± 0.344	12.318 ± 0.210	12.390 ± 0.505
Heart							
Absolute	0.57 ± 0.01	0.53 ± 0.01	0.57 ± 0.02	0.51 ± 0.02	0.54 ± 0.01	0.53 ± 0.01	0.54 ± 0.02
Relative	3.921 ± 0.083	3.766 ± 0.063	3.892 ± 0.081	3.664 ± 0.072	3.817 ± 0.044	3.685 ± 0.044	3.775 ± 0.091
R. Kidney							
Absolute	0.69 ± 0.02	0.66 ± 0.01	0.67 ± 0.02	0.65 ± 0.02	0.64 ± 0.02	0.65 ± 0.01	0.63 ± 0.02
Relative	4.738 ± 0.181	4.693 ± 0.115	4.642 ± 0.075	4.653 ± 0.168	4.540 ± 0.121	4.528 ± 0.088	4.395 ± 0.053
Liver							
Absolute	6.28 ± 0.22	6.17 ± 0.16	6.62 ± 0.24	6.90 ± 0.20	7.44 ± 0.22**	7.71 ± 0.10**	8.55 ± 0.31**
Relative	43.297 ± 1.032	43.914 ± 0.757	45.616 ± 0.915	49.491 ± 0.616**	52.406 ± 1.081**	54.089 ± 0.800**	60.068 ± 1.475**
Lung							
Absolute	0.90 ± 0.03	087 ± 0.01	0.92 ± 0.03	0.84 ± 0.05	0.86 ± 0.03	0.85 ± 0.02	0.89 ± 0.05
Relative	6.212 ± 0.233	6.166 ± 0.055	6.326 ± 0.204	6.022 ± 0.250	6.052 ± 0.127	5.937 ± 0.121	6.243 ± 0.296
Thymus							
Absolute	0.438 ± 0.019	0.403 ± 0.013	0.396 ± 0.015	0.389 ± 0.025	0.386 ± 0.019	0.391 ± 0.011	0.409 ± 0.032
Relative	3.035 ± 0.201	2.868 ± 0.089	2.731 ± 0.057	2.776 ± 0.095	2.715 ± 0.119	2.744 ± 0.087	2.865 ± 0.197

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

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Male						
n	5	10	10	10	10	10
Necropsy body wt	32.4 ± 7	330 ± 10	336 ± 12	339 ± 6	328 ± 6	299 ± 7*
Heart						
Absolute	0.84 ± 0.02	0.82 ± 0.03	0.87 ± 0.03	0.85 ± 0.01	0.86 ± 0.02	0.84 ± 0.02
Relative	2.604 ± 0.022	2.494 ± 0.021	2.593 ± 0.017	2.517 ± 0.030	2.633 ± 0.034	2.789 ± 0.029**
R. Kidney						
Absolute	0.99 ± 0.03	1.00 ± 0.03	1.04 ± 0.04	1.02 ± 0.02	1.05 ± 0.03	1.02 ± 0.03
Relative	3.059 ± 0.057	3.017 ± 0.032	3.097 ± 0.045	3.010 ± 0.028	3.197 ± 0.032	3.392 ± 0.046**
Liver						
Absolute	10.56 ± 0.36	11.61 ± 0.56	12.58 ± 0.44**	13.68 ± 0.26**	14.47 ± 0.34**	14.76 ± 0.39**
Relative	32.554 ± 0.486	35.014 ± 0.751**	37.527 ± 0.308**	40.383 ± 0.512**	44.151 ± 0.415**	49.331 ± 0.546**
Lung						
Absolute	1.34 ± 0.04	1.38 ± 0.05	1.47 ± 0.06	1.37 ± 0.03	1.41 ± 0.06	1.26 ± 0.03
Relative	4.146 ± 0.119	4.186 ± 0.084	4.399 ± 0.127	4.035 ± 0.098	4.289 ± 0.112	4.218 ± 0.119
R. Testis						
Absolute	1.395 ± 0.022	1.380 ± 0.036[b]	1.447 ± 0.043	1.404 ± 0.029	1.431 ± 0.026	1.372 ± 0.027
Relative	4.311 ± 0.074	4.189 ± 0.120	4.327 ± 0.065	4.145 ± 0.069	4.371 ± 0.040	4.593 ± 0.057*
Thymus						
Absolute	0.238 ± 0.010	0.241 ± 0.007	0.246 ± 0.008	0.255 ± 0.007	0.243 ± 0.007	0.207 ± 0.006
Relative	0.733 ± 0.027	0.732 ± 0.017	0.737 ± 0.022	0.752 ± 0.019	0.746 ± 0.026	0.692 ± 0.015
Female						
n	10	10	10	10	10	10
Necropsy body wt	177 ± 4	183 ± 5	175 ± 7	173 ± 2	174 ± 3	167 ± 3
Heart						
Absolute	0.54 ± 0.02	0.56 ± 0.01	0.54 ± 0.02	0.54 ± 0.01	0.56 ± 0.01	0.55 ± 0.01
Relative	3.046 ± 0.038	3.044 ± 0.039	3.105 ± 0.096	3.105 ± 0.032	3.238 ± 0.041*	3.305 ± 0.032**
R. Kidney						
Absolute	0.64 ± 0.02	0.68 ± 0.01	0.64 ± 0.02	0.66 ± 0.01	0.68 ± 0.01	0.70 ± 0.02
Relative	3.633± 0.051	3.693 ± 0.040	3.679 ± 0.076	3.820 ± 0.043*	3.930 ± 0.063**	4.156 ± 0.081**
Liver						
Absolute	5.32 ± 0.20	6.42 ± 0.18**	6.15 ± 0.33**	6.83 ± 0.12**	7.73 ± 0.12**	8.46 ± 0.24**
Relative	29.978 ± 0.611	35.097 ± 0.501**	35.099 ± 0.915**	39.528 ± 0.607**	44.530 ± 0.633**	50.535 ± 0.920**
Lung						
Absolute	0.89 ± 0.03	0.94 ± 0.02	0.91 ± 0.04	0.88 ± 0.02	0.95 ± 0.02	0.92 ± 0.02
Relative	5.042 ± 0.112	5.169 ± 0.104	5.213 ± 0.152	5.070 ± 0.104	5.461 ± 0.105*	5.490 ± 0.077**
Thymus						
Absolute	0.196 ± 0.008	0.198 ± 0.007	0.178 ± 0.014	0.198 ± 0.007	0.190 ± 0.005	0.198 ± 0.005
Relative	1.106 ± 0.044	1.081 ± 0.038	1.009 ± 0.059	1.146 ± 0.037	1.096 ± 0.033	1.184 ± 0.027

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

	0 ppm	1,560 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
n	5	5	5	5	5	5	5
Male							
Necropsy body wt	22.5 ± 0.4	22.3 ± 0.4	22.7 ± 0.5	22.3 ± 0.2	21.7 ± 0.4	22.5 ± 0.2	20.9 ± 0.6
Brain							
Absolute	0.469 ± 0.020	0.489 ± 0.012	0.475 ± 0.019	0.469 ± 0.010	0.460 ± 0.012	0.471 ± 0.012	0.439 ± 0.007
Relative	20.871 ± 0.663	21.927 ± 0.367	21.021 ± 1.087	21.042 ± 0.446	21.255 ± 0.315	20.997 ± 0.576	21.022 ± 0.541
Heart							
Absolute	0.12 ± 0.00	0.12 ± 0.01	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.01	0.12 ± 0.00	0.11 ± 0.00
Relative	5.159 ± 0.051	5.399 ± 0.150	5.393 ± 0.138	5.391 ± 0.168	5.197 ± 0.264	5.136 ± 0.118	5.452 ± 0.093
R. Kidney							
Absolute	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.02	0.23 ± 0.01	0.21 ± 0.01
Relative	10.551 ± 0.257	10.893 ± 0.248	10.457 ± 0.423	10.487 ± 0.331	10.042 ± 0.549	10.368 ± 0.378	10.159 ± 0.054
Liver							
Absolute	1.23 ± 0.03	1.22 ± 0.04	1.26 ± 0.05	1.29 ± 0.04	1.29 ± 0.06	1.51 ± 0.01**	1.67 ± 0.07**
Relative	54.872 ± 1.270	54.480 ± 1.301	55.369 ± 1.737	58.038 ± 1.902	59.334 ± 1.904	67.375 ± 0.725**	79.792 ± 1.992**
Lung							
Absolute	0.16 ± 0.00	0.17 ± 0.00	0.16 ± 0.00	0.17 ± 0.00*	0.15 ± 0.00	0.16 ± 0.00	0.15 ± 0.01
Relative	7.117 ± 0.192	7.399 ± 0.095	7.081 ± 0.112	7.794 ± 0.120*	6.955 ± 0.123	7.062 ± 0.186	6.996 ± 0.132
R. Testis							
Absolute	0.104 ± 0.004	0.107 ± 0.003	0.104 ± 0.002	0.103 ± 0.001	0.106 ± 0.003	0.102 ± 0.001	0.101 ± 0.004
Relative	4.635 ± 0.113	4.776 ± 0.102	4.597 ± 0.126	4.632 ± 0.027	4.892 ± 0.105	4.534 ± 0.064	4.802 ± 0.084
Thymus							
Absolute	0.060 ± 0.005	0.048 ± 0.003	0.059 ± 0.006	0.050 ± 0.005	0.042 ± 0.002*	0.052 ± 0.003*	0.040 ± 0.001**
Relative	2.697 ± 0.288	2.162 ± 0.121	2.571 ± 0.214	2.253 ± 0.244	1.937 ± 0.099*	2.321 ± 0.147	1.909 ± 0.093*
Female							
Necropsy body wt	19.4 ± 0.4	19.4 ± 0.4	19.2 ± 0.4	20.0 ± 0.3	19.2 ± 0.2	19.3 ± 0.6	19.2 ± 0.3
Brain							
Absolute	0.494 ± 0.014	0.478 ± 0.008	0.487 ± 0.013	0.475 ± 0.008	0.472 ± 0.016	0.474 ± 0.017	0.488 ± 0.015
Relative	25.433 ± 0.538	24.712 ± 0.572	25.422 ± 0.509	23.750 ± 0.170	24.548 ± 0.701	24.615 ± 0.293	25.407 ± 0.889
Heart							
Absolute	0.10 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.01	0.10 ± 0.00
Relative	5.377 ± 0.164	5.508 ± 0.094	5.587 ± 0.124	5.224 ± 0.110	5.173 ± 0.103	5.315 ± 0.156	5.127 ± 0.128
R. Kidney							
Absolute	0.15 ± 0.00	0.16 ± 0.01	0.16 ± 0.00	0.16 ± 0.00	0.15 ± 0.00	0.15 ± 0.01	0.15 ± 0.01
Relative	7.852 ± 0.068	8.118 ± 0.257	8.390 ± 0.112	7.979 ± 0.214	7.615 ± 0.161	7.612 ± 0.198	7.856 ± 0.269
Liver							
Absolute	1.02 ± 0.03	1.02 ± 0.04	0.94 ± 0.04	1.10 ± 0.03	0.98 ± 0.02	1.03 ± 0.04	1.21 ± 0.04**
Relative	52.640 ± 0.825	52.343 ± 1.001	48.980 ± 0.901	55.120 ± 1.299	50.860 ± 0.606	53.669 ± 0.642	62.749 ± 1.548**
Lung							
Absolute	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.00	0.16 ± 0.01	0.16 ± 0.01	0.14 ± 0.00	0.15 ± 0.01
Relative	7.923 ± 0.439	8.174 ± 0.416	7.780 ± 0.145	8.026 ± 0.169	8.093 ± 0.337	7.264 ± 0.119	7.875 ± 0.403
Thymus							
Absolute	0.079 ± 0.005	0.070 ± 0.004	0.067 ± 0.004	0.070 ± 0.002	0.066 ± 0.005	0.062 ± 0.008	0.075 ± 0.005
Relative	4.048 ± 0.247	3.585 ± 0.173	3.481 ± 0.232	3.492 ± 0.107	3.435 ± 0.271	3.187 ± 0.363	3.894 ± 0.303

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

Table G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Feed Study of Goldenseal Root Powder[a]

	0 ppm	3,121 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	32.4 ± 0.6	31.7 ± 0.8	32.0 ± 0.5	32.8 ± 0.6	31.6 ± 0.5	29.9 ± 0.5
Heart						
Absolute	0.15 ± 0.00	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.00	0.16 ± 0.01	0.15 ± 0.01
Relative	4.555 ± 0.123	4.832 ± 0.165	4.822 ± 0.124	4.703 ± 0.074	5.052 ± 0.199*	5.055 ± 0.171*
R. Kidney						
Absolute	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.00	0.29 ± 0.01*	0.27 ± 0.01	0.25 ± 0.00
Relative	8.328 ± 0.164	8.570 ± 0.173	8.524 ± 0.127	8.984 ± 0.197	8.552 ± 0.203	8.487 ± 0.203
Liver						
Absolute	1.38 ± 0.03	1.43 ± 0.07	1.46 ± 0.04	1.57 ± 0.03**	1.68 ± 0.05**	1.97 ± 0.05**
Relative	42.790 ± 1.000	45.021 ± 1.473	45.684 ± 1.097	47.993 ± 1.056**	52.948 ± 1.090**	65.822 ± 0.977**
Lung						
Absolute	0.22 ± 0.01	0.23 ± 0.02	0.23 ± 0.01	0.24 ± 0.02	0.23 ± 0.02	0.26 ± 0.01
Relative	6.899 ± 0.476	7.271 ± 0.509	7.167 ± 0.465	7.288 ± 0.503	7.366 ± 0.397	8.727 ± 0.301*
R. Testis						
Absolute	0.126 ± 0.007	0.128 ± 0.008	0.128 ± 0.006	0.124 ± 0.003	0.130 ± 0.010	0.121 ± 0.002
Relative	3.880 ± 0.212	4.088 ± 0.303	3.998 ± 0.195	3.774 ± 0.068	4.111 ± 0.308	4.064 ± 0.059
Thymus						
Absolute	0.033 ± 0.001	0.030 ± 0.002	0.029 ± 0.002	0.031 ± 0.001	0.029 ± 0.002	0.032 ± 0.001
Relative	1.020 ± 0.039	0.944 ± 0.029	0.915 ± 0.071	0.959 ± 0.032	0.916 ± 0.077	1.060 ± 0.035
Female						
Necropsy body wt	26.9 ± 0.7	27.3 ± 0.7	27.6 ± 0.7	26.3 ± 0.3	25.1 ± 0.2*	22.9 ± 0.5**
Heart						
Absolute	0.12 ± 0.00	0.11 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.00**
Relative	4.474 ± 0.083	4.101 ± 0.061	4.259 ± 0.099	4.384 ± 0.137	4.700 ± 0.104	4.592 ± 0.113
R. Kidney						
Absolute	0.16 ± 0.00	0.17 ± 0.00	0.17 ± 0.00	0.16 ± 0.00	0.17 ± 0.01	0.15 ± 0.00*
Relative	6.131 ± 0.224	6.097 ± 0.166	6.302 ± 0.197	6.204 ± 0.142	6.689 ± 0.209	6.378 ± 0.098
Liver						
Absolute	1.23 ± 0.03	1.22 ± 0.02	1.27 ± 0.03	1.22 ± 0.02	1.37 ± 0.05**	1.37 ± 0.06**
Relative	45.927 ± 1.578	44.685 ± 0.878	45.974 ± 0.749	46.510 ± 0.700	54.586 ± 2.023**	59.694 ± 1.327**
Lung						
Absolute	0.24 ± 0.02	0.20 ± 0.02	0.24 ± 0.02	0.24 ± 0.01	0.24 ± 0.02	0.25 ± 0.04
Relative	9.140 ± 0.604	7.455 ± 0.520	8.587 ± 0.606	9.033 ± 0.438	9.655 ± 0.635	11.133 ± 1.804
Thymus						
Absolute	0.027 ± 0.002	0.034 ± 0.002	0.032 ± 0.002	0.036 ± 0.002*	0.031 ± 0.002	0.034 ± 0.001
Relative	1.016 ± 0.048	1.234 ± 0.064	1.157 ± 0.069	1.372 ± 0.086**	1.231 ± 0.076**	1.502 ± 0.067**

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

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

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TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Feed Study of Goldenseal Root Powder[a]

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	324 ± 7	339 ± 6	328 ± 6	299 ± 7*
L. Cauda epididymis	0.2054 ± 0.0090	0.2037 ± 0.0054	0.2100 ± 0.0057	0.1979 ± 0.0046
L. Epididymis	0.4534 ± 0.0156	0.4831 ± 0.0065	0.4782 ± 0.0135	0.4548 ± 0.0113
L. Testis	1.4983 ± 0.0262	1.5375 ± 0.0303	1.5475 ± 0.0305	1.4930 ± 0.0305
Spermatid measurements				
Spermatid heads (10^6/g testis)	126.42 ± 7.91	129.62 ± 3.35	119.67 ± 3.69	120.02 ± 3.85
Spermatid heads (10^6/testis)	171.75 ± 9.76	173.50 ± 5.13	161.63 ± 3.50	157.88 ± 4.86
Epididymal spermatozoal measurements				
Sperm (10^6/g cauda epididymis)	503 ± 29	523 ± 29	495 ± 25	550 ± 35
Sperm (10^6/cauda epididymis)	102 ± 5	106 ± 6	103 ± 5	108 ± 6
Sperm motility (%)	66.2 ± 1.2	66.5 ± 1.3	63.3 ± 1.1	63.5 ± 1.1

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Data are presented as mean ± standard error. Differences from the 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 Feed Study of Goldenseal Root Powder[a]

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	177 ± 4	173 ± 2	174 ± 3	167 ± 3*
Proportion of regular cycling females[b]	10/10	9/10	9/10	7/10
Estrous cycle length (days)	4.8 ± 0.2	4.6 ± 0.3[c]	4.9 ± 0.1	5.4 ± 0.2[c]
Estrous stages (% of cycle)				
Diestrus	61.9	67.0	64.7	61.3
Proestrus	10.6	12.2	15.5	13.4
Estrus	23.0	19.1	19.8	19.3
Metestrus	4.4	1.7	0.0	5.9

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Feed Study of Goldenseal Root Powder[a]

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	32.4 ± 0.8	32.8 ± 0.6	31.6 ± 0.5	29.9 ± 0.4**
L. Cauda epididymis	0.0286 ± 0.0016	0.0312 ± 0.0018	0.0282 ± 0.0016	0.0245 ± 0.0006
L. Epididymis	0.0615 ± 0.0016	0.0623 ± 0.0016	0.0590 ± 0.0022	0.0524 ± 0.0006**
L. Testis	0.1204 ± 0.0012	0.1248 ± 0.0034	0.1202 ± 0.0020	0.1236 ± 0.0018
Spermatid measurements				
Spermatid heads (10^6/g testis)	171.70 ± 9.62	187.28 ± 7.57	179.52 ± 3.60	187.28 ± 5.64
Spermatid heads (10^6/testis)	19.61 ± 1.16	21.20 ± 1.07	19.56 ± 0.39	21.32 ± 0.75
Epididymal spermatozoal measurements				
Sperm (10^6/g cauda epididymis)	673 ± 39	637 ± 35	732 ± 55	749 ± 31
Sperm (10^6/cauda epididymis)	19 ± 1	20 ± 1	20 ± 1	18 ± 1
Sperm motility (%)	64.4 ± 1.5	64.1 ± 2.5	59.6 ± 1.4	60.9 ± 1.7

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

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Feed Study of Goldenseal Root Powder[a]

	0 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Number weighted at necropsy	10	10	10	10
Necropys body wt (g)	26.9 ± 0.7	26.3 ± 0.3	25.1 ± 0.2**	22.9 ± 0.5**
Proportion of regular cycling females[b]	6/10	4/10	7/10	9/10
Estrous cycle length (days)	4.7 ± 0.5	4.9 ± 0.4	4.2 ± 0.4	4.6 ± 0.5
Estrous stages (% of cycle)				
Diestrus	39.8	45.6	47.7	39.2
Proestrus	0.0	0.0	0.0	0.0
Estrus	43.7	40.4	40.5	39.2
Metestrus	16.5	14.0	11.7	21.7

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Goldenseal Root Powder

Goldenseal root powder was obtained from Plantation Medicinals, Inc. (Felda, FL), in one lot (007-090200) used for the 2-week and 3-month studies. Goldenseal roots were purchased from Strategic Sourcing, Inc. (Reading, PA), in one lot (HYCA 10/7-10.28.01-C) that was used for the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Midwest Research Institute (Kansas City, MO) and by the study laboratories at BioReliance Corporation (Rockville, MD; 2-week studies) and Southern Research Institute (Birmingham, AL; 3-month and 2-year studies). Nutritional, contaminant, and microbiological tests were conducted by Covance Laboratories, Inc. (Madison, WI). Stability analyses of the bulk chemical were conducted by the analytical chemistry laboratory. Reports on analyses performed in support of the goldenseal root powder studies are on file at the National Institute of Environmental Health Sciences.

Goldenseal root powder (lot 007-090200) was identified by its color and alkaloid profile as described in the literature (AHP, 2001). Goldenseal root (HYCA10/7-10.28.01C) was also identified by its distinctive appearance prior to grinding. Characteristically, goldenseal root powder contains no less than 2.0% hydrastine and 2.5% berberine on a dry weight basis. Both lots were milled so that at least 75% of the resulting material passed through a 60-mesh (250 µm) sieve. Identity was further confirmed from the profile and presence of the alkaloids berberine, hydrastine, and canadine together, which is characteristic of goldenseal root, and the absence of palmatine, which is characteristic of coptis, a common adulterant of goldenseal products.

The purities of acetonitrile:water:phosphoric acid (70:30:0.1) extracts of lots 007-090200 and HYCA 10/7-10.28.01-C were determined by the analytical chemistry laboratory and confirmed by the study laboratories using high-performance liquid chromatography (HPLC) by system A.

A) Waters 2690 chromatograph (Waters Corporation, Milford, MA) or equivalent, a ZORBAX® Eclipse XDB-C18 column (150 mm × 4.6 mm, 5-µm particle size; Agilent Technologies, Palo Alto, CA), an isocratic mobile phase of either 68:32 or 72:28 buffer:acetonitrile, a flow rate of 1.0 mL/minute, and ultraviolet detection at 320 nm. The buffer component of the mobile phase consisted of 30 mM ammonium acetate and 14 mM triethylamine at a pH of approximately 4.85 (adjusted with acetic acid).

The purity of lot HYCA 10/7-10.28.01-C was also determined by the analytical chemistry laboratory using thin-layer chromatography (TLC), headspace and extract analyses by gas chromatography coupled with mass spectrometry (GC/MS), liquid chromatography coupled with mass spectrometry (LC/MS), and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF/MS).

TLC was performed on silica gel 60, F_{254} plates with a 200-µm layer thickness (E.M. Science, Gibbstown, NJ). Samples of the test article were extracted with a solvent consisting of acetonitrile:water:phosphoric acid (70:30:0.1) and the extracts were diluted 50:50 with methanol and spotted alongside standard methanol solutions of hydrastinine, hydrastine, berberine, and palmatine. The spotted plates were developed in a chamber containing ethyl acetate:methanol:formic acid:water (50:10:6:3), dried, and visualized at 254 nm and 366 nm.

GC/MS analysis of headspace volatile organic components in the test article was conducted on trapped air samples collected from vials of goldenseal root powder heated to 110° C for 2 hours. The GC/MS system utilized a Fisons 8035 gas chromatograph coupled to a Fisons MD800 mass spectrometer (Fisons Instruments, Beverly, MA), a DB-5 MS column (30 m × 0.32 mm, 1-µm film thickness; J&W Scientific, Folsom, CA), helium carrier gas at 4 psi head pressure, and an oven temperature program of 40° C for 4 minutes, a 10° C/minute increase to 300° C, and a 20-minute hold at 300° C. The mass spectrometer was operated in electron impact mode with an ionization

energy of 70 eV and scanned a mass range from 35 to 450 atomic mass units (amu). Hexane, dichloromethane, and acetone extracts of the bulk chemical were analyzed with this same GC/MS system to measure volatile and semivolatile components in the test article.

LC/MS analyses of nonvolatile constituents of water, methanol, or acetonitrile:water:trifluoroacetic acid (70:30:0.1) extracts of the test article were conducted using HPLC by system A. The mass spectra for these analyses (scanned from 150 to 600 amu) were obtained on a Fisons Quattro I mass spectrometer (Fisons Instruments) operated in electrospray ionization mode.

MALDI-TOF/MS analysis was conducted on water, methanol, and acetonitrile:water:phosphoric acid (70:30:01) extracts of the test article using an Applied Biosystems Voyager-DE^{TM} STR mass spectrometer (Applied Biosystems, Foster City, CA). The analytical system was equipped with a nitrogen laser (337 nm) and was operated in reflector and linear modes.

The analytical chemistry laboratory determined that lot 007-090200 contained 3.45% berberine, 3.02% hydrastine, and 0.08% canadine by weight; palmatine was not detected. An aliquot of lot 007-090200 was submitted to Covance Laboratories, Inc. for nutritional and contaminant testing using standard methods. Organochlorine and organophosphorus pesticide levels were below the detection limit except for captan, which was present at 113 ppb. Aflatoxin and nitrosamine levels were below the detection limit of 1 ppb and mercury was below the detection limit of 25 ppb. Lead and selenium were present at 204 and 3.8 ppb, respectively. Microbial tests were not performed on lot 007-090200.

For lot HYCA 10/7-10.28.01-C, weight loss on drying indicated a moisture content of 6.35%. Hydrastinine, hydrastine, and berberine were visualized by TLC, but palmatine was not observed. HPLC analyses by the analytical chemistry laboratory using the same system indicated that palmatine, berberine, hydrastine, canadine, and total alkaloids were present at 0.0%, 3.89%, 2.80%, 0.17%, and 6.86%, respectively, and the B/H ratio was determined to be 2.84. By comparison with a mass spectral library, 17 volatile organic components were tentatively identified by GC/MS in the goldenseal root powder headspace samples and five components (including hydrastinine, hydrastine, and canadine) were tentatively identified in the test article extracts. LC/MS analyses of goldenseal root powder extracts did not detect palmatine but did identify hydrastinine, hydrastine, berberine, and canadine, as well as the known goldenseal alkaloids tetrahydroberberastine, canadaline, and berberastine. MALDI-TOF/MS analyses detected ions corresponding to hydrastine, berberine, and several unidentified components in all of the goldenseal extracts and ions corresponding to hydrastidine and/or canadaline only in the acetonitrile:water:phosphoric acid (70:30:0.1) extract. An aliquot of lot HYCA 10/7-10.278.91-C was submitted to Covance Laboratories, Inc. for nutritional and contaminant testing using standard methods. Organochlorine and organonphosphorus pesticide levels were below the detection limit, except for pentachloronitrobenzene (PCNB), which was present at 218 ppb. Lead, arsenic, and selenium were present at 450, 130, and 45 ppb, respectively. Mercury levels were below the detection limit of 25 ppb. Aflatoxin and nitrosamine levels were below the detection limit of 1 ppb. Microbial testing results were as follow: standard plate count, 11,000 CFU/g; total coliform, 43 MPN/g; *Salmonella typhimurium*, negative per 25g; mold count, 35 col/g; fecal coliform less than 3.0 MPN/g, *Escherichia coli* less than 10 CFU/g; and yeast count, less than 10 col/g. Results of the nutritional, contaminant, and microbiological tests were deemed acceptable for use in these studies.

Stability studies of lot HYCA 10/7-10.28.01-C were performed by the analytical chemistry laboratory by monitoring the palmatine, berberine, hydrastine, canadine, and total alkaloids content of the test article. Samples of the bulk chemical were extracted with acetonitrile:water:phosphoric acid (70:30:0.1) and the extracts were analyzed by HPLC using system A. These studies indicated that goldenseal root powder was stable as a bulk chemical for at least 2 weeks when stored protected from light at temperatures up to 25° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in plastic bags. Periodic reanalyses of the bulk chemical were performed by the study laboratories using HPLC by system A; no degradation of the bulk chemical was detected by measuring the B/H area ratios.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing goldenseal root powder with feed (Table I1). Formulations were stored in double-thickness plastic bags, protected from light at 2° C to 8° C for up to 35 days.

Homogeneity studies of 1,560 and 50,000 ppm dose formulations and of 3,121 and 50,000 ppm dose formulations were performed by BioReliance and Southern Research Institute, respectively. These studies were conducted with HPLC by system A and measured the berberine content of acetonitrile:water:phosphoric acid (70:30:0.1) extracts of the dose formulations. These analytical procedures were also used in stability studies of a 1,500 ppm dose formulation of lot 8147 that were performed by the analytical chemistry laboratory. Homogeneity was confirmed, and stability was confirmed for at least 35 days for dose formulations stored in opaque double-thickness plastic bags under freezer, refrigerated, and room temperature conditions; stability was also confirmed for at least 17 days under simulated animal room conditions.

Periodic analyses of the dose formulations of goldenseal root powder were conducted by the study laboratories using HPLC by system A. All determinations of the concentrations of goldenseal root powder in feed were based on quantification of peak areas produced by the marker compound berberine. During the 2-week studies, the dose formulations were analyzed once; all six dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table I2). Animal room samples of these dose formulations were also analyzed; four of six for rats and one of six for mice were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed at the beginning and at the end of the studies; animal room samples were also analyzed (Table I3). All 40 dose formulations analyzed for rats and mice were within 10% of the target concentrations; all 10 animal room samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 8 to 12 weeks (Table I4). Of the dose formulations analyzed, all 173 for rats and all 85 for mice were within 10% of the target concentrations; all 15 animal room samples for rats and 11 of 12 for mice were within 10% of the target concentrations.

TABLE I1
Preparation and Storage of Dose Formulations in the Feed Studies of Goldenseal Root Powder

2-Week Studies	3-Month Studies	2-Year Studies
Preparation		
A premix of feed and goldenseal root powder was prepared, then layered into the remaining feed and blended in a Patterson-Kelly twin-shell blender with the intensifier bar on for 5 minutes and off for 10 minutes. The dose formulations were prepared once during the studies.	A premix of feed and goldenseal root powder was prepared, then layered into the remaining feed and blended for 30 minutes in a Patterson-Kelly twin-shell blender with the intensifier bar on for the entire time. The dose formulations were prepared four times.	A premix of feed and goldenseal root powder was prepared, then layered into the remaining feed and blended for 30 minutes (or 15 minutes for dose formulations prepared after April 10, 2003) in a Patterson-Kelly twin-shell blender with the intensifier bar on for the entire time. The dose formulations were prepared approximately monthly.
Chemical Lot Number		
007-090200	007-090200	HYCA 10/7-10.28.01-C
Maximum Storage Time		
35 days	35 days	35 days
Storage Conditions		
Stored protected from light, in double-thickness plastic bags, at 2° C to 8° C	Stored protected from light, in double-thickness plastic bags, at 2° C to 8° C	Stored protected from light, in double-thickness plastic bags, at 2° C to 8° C
Study Laboratory		
BioReliance Corporation (Rockville, MD)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Feed Studies of Goldenseal Root Powder[a]

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[b] (ppm)	Difference from Target (%)
June 13, 2001	June 14, 2001	1,560	1,670	+7
		3,121	3,060	–2
		6,250	6,510	+4
		12,500	12,400	–1
		25,000	24,400	–2
		50,000	49,200	–2
	July 18, 2001[c]	1,560	1,310	–16
		3,121	2,510	–20
		6,250	6,100	–2
		12,500	12,200	–2
		25,000	23,500	–6
		50,000	46,500	–7
	July 18, 2001[d]	1,560	1,310	–16
		3,121	2,470	–21
		6,250	5,690	–9
		12,500	20,300	+62
		25,000	22,300	–11
		50,000	42,200	–16

[a] Target and determined concentrations express measured concentrations of berberine.
[b] Results of duplicate analyses
[c] Animal room samples for rats
[d] Animal room samples for mice

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Feed Studies of Goldenseal Root Powder[a]

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[b] (ppm)	Difference from Target (%)
January 16, 2002	January 17-18, 2002	3,121	3,215	+3
		3,121	3,185	+2
		3,121	3,203	+2
		6,250	6,388	+2
		6,250	6,399	+2
		6,250	6,374	+2
		12,500	12,325	–1
		12,500	12,607	+1
		12,500	12,461	0
		25,000	25,174	+1
		25,000	24,877	0
		25,000	25,281	+1
		50,000	50,583	+1
		50,000	50,405	+1
		50,000	49,912	0
	February 22, 2002[d]	3,121	3,172	+2
		6,250	6,218	–1
		12,500	12,198	–2
		25,000	25,075	0
		50,000	50,480	+1
February 13, 2002	February 14-15, 2002	3,121	3,324	+6
		3,121	3,302	+6
		6,250	6,439	+3
		6,250	6,459	+3
		12,500	12,810	+2
		12,500	13,045	+4
		25,000	25,768	+3
		25,000	25,831	+3
		50,000	51,014	+2
		50,000	51,705	+3
	March 22-23, 2002[c]	3,121	3,034	–3
		6,250	6,221	0
		12,500	12,264	–2
		25,000	24,239	–3
		50,000	49,555	–1
	March 22-23, 2002[d]	3,121	3,035	–3
		6,250	6,117	–2
		12,500	11,917	–5
		25,000	23,686	–5
		50,000	47,105	–6

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Feed Studies of Goldenseal Root Powder

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
April 5, 2002	April 8-9, 2002	3,121	3,145	+1
		3,121	3,122	0
		3,121	3,139	0
		6,250	6,262	0
		6,250	6,258	0
		6,250	6,297	+1
		12,500	12,538	0
		12,500	12,390	–1
		12,500	12,416	–1
		25,000	24,763	–1
		25,000	24,963	0
		25,000	25,101	0
		50,000	49,979	0
		50,000	50,252	+1
		50,000	50,290	+1
	May 6-7, 2002[c]	3,121	3,095	–1
		6,250	6,001	–4
		12,500	12,121	–3
		25,000	24,658	–1
		50,000	49,764	0
	May 6-7, 2002[d]	3,121	3,070	–2
		6,250	6,112	–2
		12,500	12,107	–3
		25,000	23,589	–6
		50,000	48,625	–3

[a] Target and determined concentrations express measured concentrations of berberine.
[b] Results of duplicate analyses
[c] Animal room samples for rats
[d] Animal room samples for mice

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Goldenseal Root Powder[a]

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[b] (ppm)	Difference from Target (%)
Rats				
April 9-10, 2003	April 10-14, 2003	3,000	3,140	+5
		3,000	2,917	–3
		3,000	3,086	+3
		3,000	3,094	+3
		3,000	3,050	+2
		3,000	3,014	0
		3,000	3,286	+10
		9,000	8,862	–2
		9,000	8,836	–2
		9,000	8,953	–1
		9,000	9,078	+1
		9,000	9,148	+2
		9,000	9,347	+4
		9,000	9,054	+1
		25,000	24,776	–1
		25,000	24,063	–4
		25,000	24,360	–3
		25,000	24,260	–3
		25,000	24,656	–1
		25,000	24,108	–4
		25,000	24,734	–1
	May 12, 2003[c]	3,000	2,815	–6
		9,000	8,987	0
		25,000	24,506	–2
April 23-25, 2003	April 24-28, 2003	3,000	3,043	+1
		3,000	2,881	–4
		3,000	2,941	–2
		3,000	2,979	–1
		9,000	9,009	0
		9,000	9,488	+5
		9,000	8,883	–1
		9,000	8,865	–2
		25,000	23,500	–6
		25,000	23,762	–5
		25,000	23,851	–5
		25,000	24,835	–1
	May 22-23, 2003[c]	3,000	2,808	–6
		9,000	8,778	–2
		25,000	24,684	–1

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Goldenseal Root Powder

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
July 2, 2003	July 7-8, 2003	3,000	3,314	+10
		3,000	2,943	–2
		3,000	2,885	–4
		3,000	2,963	–1
		3,000	2,911	–3
		9,000	9,066	+1
		9,000	8,893	–1
		9,000	8,739	–3
		9,000	8,801	–2
		9,000	9,257	+3
		25,000	24,205	–3
		25,000	24,811	–1
		25,000	24,931	0
		25,000	26,173	+5
		25,000	23,845	–5
September 10, 2003	September 11-12, 2003	3,000	3,013	0
		3,000	2,912	–3
		3,000	2,943	–2
		3,000	3,126	+4
		9,000	8,850	–2
		9,000	9,064	+1
		9,000	8,600	–4
		9,000	8,557	–5
		25,000	23,881	–4
		25,000	24,621	–2
		25,000	24,176	–3
		25,000	24,373	–3
September 16, 2003	September 16, 2003	3,000	2,978[d]	–1
November 19-20, 2003	November 20-21, 2003	3,000	2,999	0
		3,000	3,062	+2
		3,000	2,998	0
		3,000	3,045	+2
		3,000	3,020	+1
		9,000	8,679	–4
		9,000	8,942	–1
		9,000	8,817	–2
		9,000	8,687	–3
		9,000	8,896	–1
		25,000	24,937	0
		25,000	24,462	–2
		25,000	24,678	–1
		25,000	24,623	–2
	December 18-19, 2003[c]	3,000	3,083	+3
		9,000	9,061	+1
		25,000	25,518	+2

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Goldenseal Root Powder

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
January 28, 2004	January 29-30, 2004	3,000	3,055	+2
		3,000	3,212	+7
		3,000	3,030	+1
		3,000	3,101	+3
		3,000	2,818	–6
		9,000	8,871	–1
		9,000	8,846	–2
		9,000	8,917	–1
		9,000	9,077	+1
		25,000	25,770	+3
		25,000	25,349	+1
		25,000	25,415	+2
		25,000	25,030	0
		25,000	25,546	+2
April 7-8, 2004	April 8-12, 2004	3,000	3,137	+5
		3,000	2,994	0
		3,000	3,006	0
		3,000	3,164	+5
		3,000	3,159	+5
		9,000	9,591	+7
		9,000	9,284	+3
		9,000	9,413	+5
		9,000	9,094	+1
		9,000	8,818	–2
		25,000	25,711	+3
		25,000	26,035	+4
		25,000	25,431	+2
		25,000	25,777	+3
		25,000	25,777	+3
		25,000	25,302	+1
June 16, 2004	June 18-21, 2004	3,000	2,926	–2
		3,000	3,018	+1
		3,000	2,738	–9
		3,000	2,941	–2
		9,000	9,119	+1
		9,000	9,042	0
		9,000	9,123	+1
		9,000	9,027	0
		9,000	9,273	+3
		25,000	24,948	0
		25,000	24,966	0
		25,000	25,197	+1
		25,000	26,124	+4
		25,000	25,316	+1
	July 21-22, 2004[c]	3,000	2,933	–2
		9,000	8,806	–2
		25,000	24,941	0
June 22, 2004	June 22, 2004	3,000	2,915[d]	–3

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Goldenseal Root Powder

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
August 25, 2004	August 26-27, 2004	3,000	2,997	0
		3,000	2,988	0
		3,000	2,964	–1
		3,000	3,013	0
		9,000	8,958	0
		9,000	9,033	0
		9,000	8,909	–1
		9,000	9,807	+9
		25,000	24,763	–1
		25,000	24,921	0
		25,000	25,408	+2
		25,000	24,294	–3
		25,000	24,963	0
November 3, 2004	November 4-5, 2004	3,000	2,904	–3
		3,000	3,067	+2
		3,000	2,958	–1
		3,000	3,035	+1
		3,000	3,000	0
		9,000	8,954	–1
		9,000	8,908	–1
		9,000	9,123	+1
		9,000	9,126	+1
		9,000	9,205	+2
		25,000	25,032	0
		25,000	25,249	+1
		25,000	25,511	+2
		25,000	24,911	0
		25,000	25,098	0
January 11, 2005	January 13-14, 2005	3,000	2,863	–5
		3,000	3,121	+4
		3,000	2,981	–1
		3,000	3,033	+1
		3,000	3,006	0
		3,000	3,099	+3
		9,000	9,338	+4
		9,000	9,190	+2
		9,000	9,613	+7
		9,000	8,940	–1
		25,000	25,255	+1
		25,000	24,062	–4
		25,000	24,425	–2
		25,000	25,043	0
	February 10-11, 2005[c]	3,000	2,830	–6
		9,000	8,555	–5
		25,000	24,197	–3

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Goldenseal Root Powder

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
March 23, 2005	March 25-26, 2005	3,000	3,045	+2
		3,000	2,874	–4
		3,000	3,006	0
		9,000	8,911	–1
		9,000	9,528	+6
		9,000	9,061	+1
		9,000	9,014	0
		25,000	24,393	–2
		25,000	24,824	–1
		25,000	24,516	–2
		25,000	24,356	–3
March 28, 2005	March 29, 2005	3,000	3,030[d]	+1
Mice				
April 23-25, 2003	April 24-28, 2003	3,000	2,973	–1
		3,000	2,881	–4
		3,000	2,978	–1
		9,000	8,897	–1
		9,000	8,865	–2
		9,000	8,256	–8
		25,000	24,835	–1
		25,000	24,484	–2
	May 22-23, 2003[c]	3,000	3,377	+13
		9,000	8,769	–3
		25,000	24,099	–4
April 29, 2003	April 29, 2003	25,000	24,825[d]	–1
July 2, 2003	July 7-8, 2003	3,000	2,885	–4
		3,000	2,963	–1
		3,000	2,899	–3
		9,000	9,066	+1
		9,000	9,367	+4
		9,000	8,739	–3
		9,000	8,801	–2
		25,000	24,811	–1
		25,000	24,931	0
		25,000	25,031	0
September 10, 2003	September 11-12, 2003	3,000	3,013	0
		3,000	2,952	–2
		9,000	8,949	–1
		9,000	8,902	–1
		9,000	8,557	–5
		25,000	24,176	–3
		25,000	24,501	–2
		25,000	24,233	–3

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Goldenseal Root Powder

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
November 19-20, 2003	November 20-21, 2003	3,000	3,111	+4
		3,000	2,999	0
		9,000	8,679	–4
		9,000	8,724	–3
		25,000	25,042	0
		25,000	24,869	–1
		25,000	24,623	–2
	December 18-19, 2003[c]	3,000	3,062	+2
		9,000	9,135	+2
		25,000	25,840	+3
January 28, 2004	January 29-30, 2004	3,000	3,055	+2
		3,000	3,212	+7
		3,000	3,030	+1
		3,000	3,101	+3
		9,000	8,871	–1
		9,000	8,917	–1
		9,000	8,960	0
		25,000	25,770	+3
		25,000	25,030	0
		25,000	25,546	+2
April 7-8, 2004	April 8-12, 2004	3,000	3,137	+5
		3,000	3,116	+4
		3,000	3,159	+5
		9,000	9,284	+3
		9,000	9,460	+5
		9,000	8,818	–2
		25,000	26,035	+4
		25,000	25,431	+2
		25,000	25,777	+3
June 16, 2004	June 18-21, 2004	3,000	3,020	+1
		3,000	2,941	–2
		9,000	8,592	–5
		9,000	9,042	0
		25,000	24,966	0
		25,000	25,542	+2
	July 21-22, 2004[c]	3,000	3,082	+3
		9,000	8,748	–3
		25,000	24,156	–3
August 25, 2004	August 26-27, 2004	3,000	2,900	–3
		3,000	2,963	–1
		9,000	8,877	–1
		9,000	9,059	+1
		25,000	24,763	–1
		25,000	25,222	+1
		25,000	24,294	–3

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Goldenseal Root Powder

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
November 3, 2004	November 4-5, 2004	3,000	2,904	–3
		3,000	2,944	–2
		9,000	9,616	+7
		9,000	9,205	+2
		25,000	25,032	0
		25,000	25,380	+2
January 11, 2005	January 13-14, 2005	3,000	2,863	–5
		3,000	3,033	+1
		3,000	3,006	0
		9,000	9,178	+2
		9,000	8,784	–2
		25,000	24,707	–1
		25,000	25,181	+1
	February 10-11, 2005[c]	3,000	3,141	+5
		9,000	9,230	+3
		25,000	23,984	–4
March 23, 2005	March 25-26, 2005	3,000	2,874	–4
		3,000	2,978	–1
		9,000	8,776	–2
		9,000	9,061	+1
		25,000	24,516	–2
		25,000	24,073	–4

[a] Target and determined concentrations express measured concentrations of berberine.
[b] Results of duplicate analyses
[c] Animal room samples
[d] Results of remix

APPENDIX J
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES OF GOLDENSEAL ROOT POWDER

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TABLE J1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Goldenseal Root Powder

Week	0 ppm		3,000 ppm			9,000 ppm			25,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ (mg/kg)
1	19.1	116	18.8	117	483	18.2	117	1,405	17.6	116	3,797
2	17.2	154	17.6	157	337	17.5	156	1,007	17.4	155	2,810
3	20.0	191	20.1	191	316	20.1	192	944	19.9	189	2,629
4	19.2	224	19.8	224	265	19.6	223	791	19.7	221	2,228
5	19.5	248	19.0	241	236	20.0	247	728	20.0	244	2,050
6	19.5	268	20.7	263	237	20.4	269	682	20.0	264	1,895
7	19.3	288	20.1	287	210	19.6	292	605	19.5	284	1,714
8	19.3	301	19.7	303	195	19.7	305	581	19.4	297	1,632
9	18.2	310	18.5	312	178	19.2	316	547	18.7	307	1,522
10	17.6	321	17.6	325	162	18.5	328	507	19.2	319	1,504
11	17.2	326	17.1	326	157	17.3	333	467	17.5	324	1,350
12	18.6	338	18.7	337	167	18.7	346	487	19.2	337	1,423
13	18.7	348	18.9	349	163	18.8	355	477	19.4	347	1,400
17	17.6	362	17.2	364	142	17.2	368	420	17.8	357	1,247
21	17.2	376	17.1	377	136	18.2	382	429	17.3	370	1,169
25	18.4	400	18.6	399	140	18.1	399	409	18.8	393	1,195
29	20.2	410	18.5	409	136	18.8	413	410	19.7	401	1,228
33	18.3	422	17.7	426	125	18.2	428	383	18.6	417	1,114
37	18.9	432	18.9	437	130	18.9	437	389	18.8	427	1,100
41	19.6	434	19.0	436	131	19.2	438	394	19.7	430	1,147
45	18.3	439	17.2	439	118	17.7	446	357	18.5	439	1,054
49	19.4	452	20.5	447	138	20.3	452	404	20.5	441	1,163
53	18.9	457	19.7	456	130	19.1	457	376	19.4	448	1,083
57	18.4	457	18.9	459	124	19.5	460	381	20.0	448	1,116
61	16.9	456	17.3	459	113	17.2	461	336	17.0	446	953
65	17.0	461	16.2	455	107	16.8	460	329	16.9	447	946
69	17.1	459	15.7	458	103	18.3	464	355	16.9	451	937
73	17.2	460	17.2	461	112	15.9	460	311	16.1	449	896
77	16.6	454	17.9	455	118	16.8	455	332	17.6	445	990
81	16.5	447	18.1	449	121	17.5	455	346	18.8	435	1,080
85	15.6	445	16.8	450	112	15.4	447	310	17.2	435	988
89	16.2	438	16.1	444	109	16.3	443	331	17.0	430	989
93	15.7	438	15.0	434	104	15.3	439	314	16.3	430	949
97	16.2	440	15.3	442	104	15.5	438	318	15.9	428	929
101	15.6	432	15.3	435	106	15.8	432	329	16.3	423	963
Mean for weeks											
1-13	18.7	264	19.0	264	239	19.0	268	710	19.0	262	1,996
14-52	18.7	414	18.3	415	133	18.5	418	399	18.9	408	1,157
53-101	16.8	450	16.9	451	113	16.9	452	336	17.3	440	986

[a] Grams of feed consumed per animal per day
[b] Milligrams of goldenseal root powder consumed per kilogram body weight per day

TABLE J2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Goldenseal Root Powder

	0 ppm		3,000 ppm			9,000 ppm			25,000 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ (mg/kg)
1	15.3	100	14.1	99	428	13.7	99	1,244	13.8	99	3,502
2	12.6	124	13.0	125	312	12.8	125	922	12.6	123	2,564
3	13.8	139	13.6	138	295	13.5	139	876	13.4	136	2,460
4	12.4	150	13.2	150	264	13.4	149	812	11.9	145	2,056
5	12.5	158	12.3	158	233	12.0	155	696	11.6	151	1,925
6	12.3	166	12.8	166	232	12.4	162	688	11.9	159	1,873
7	12.2	173	12.1	173	210	12.0	169	639	11.3	164	1,720
8	12.3	181	12.1	180	201	12.0	176	613	11.3	171	1,655
9	11.6	184	11.2	183	184	11.9	180	595	11.1	174	1,598
10	11.5	188	10.6	185	172	10.6	183	522	10.5	177	1,483
11	10.8	188	11.2	186	181	10.9	183	535	10.0	177	1,413
12	12.1	193	11.2	192	175	11.4	190	541	10.8	181	1,489
13	11.5	195	11.2	194	173	11.1	190	526	10.7	182	1,470
17	11.8	203	11.1	201	166	10.9	196	500	10.4	186	1,395
21	11.2	210	10.9	208	158	10.8	202	480	10.0	193	1,296
25	11.5	217	10.8	214	152	10.7	208	463	10.3	197	1,305
29	12.4	221	11.3	220	154	10.9	211	466	11.1	200	1,385
33	11.9	231	11.5	225	154	11.6	219	477	11.7	208	1,405
37	11.7	238	11.6	232	150	11.5	225	460	10.9	212	1,287
41	11.8	240	12.2	234	156	12.1	225	484	11.2	211	1,326
45	12.0	247	12.3	242	153	11.0	228	434	10.4	213	1,220
49	13.2	254	12.5	250	150	12.0	237	457	11.7	220	1,328
53	12.7	261	12.4	255	146	11.9	241	445	11.7	223	1,314
57	13.0	269	12.3	261	141	12.3	247	448	12.0	227	1,321
61	12.3	276	12.1	269	135	11.6	253	412	10.8	231	1,171
65	11.3	283	12.5	278	135	12.1	261	418	11.3	235	1,204
69	12.4	294	12.3	285	129	11.9	267	401	10.7	237	1,129
73	12.2	299	12.9	295	131	12.2	275	400	10.3	240	1,074
77	12.9	307	12.7	299	127	12.3	279	397	12.2	248	1,228
81	13.5	308	13.3	300	133	13.4	280	431	13.3	250	1,329
85	12.7	315	12.6	308	123	13.3	290	413	12.3	257	1,197
89	12.7	320	12.5	310	121	13.0	297	395	12.7	261	1,217
93	12.7	326	12.0	312	116	13.0	298	393	11.7	263	1,113
97	13.0	326	12.5	315	119	12.5	302	373	11.6	263	1,102
101	10.8	324	13.2	318	125	13.0	307	381	12.1	270	1,119
Mean for weeks											
1-13	12.4	165	12.2	164	235	12.1	162	708	11.6	157	1,939
14-52	11.9	229	11.6	225	155	11.3	217	469	10.9	204	1,327
53-101	12.5	301	12.6	293	129	12.5	277	408	11.7	247	1,194

[a] Grams of feed consumed per animal per day
[b] Milligrams of goldenseal root powder consumed per kilogram body weight per day

TABLE J3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of Goldenseal Root Powder

Week	0 ppm		3,000 ppm			9,000 ppm			25,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ (mg/kg)
1	7.2	20.4	7.2	20.5	1,054	7.0	20.7	3,051	7.5	20.6	9,112
2	4.9	21.1	5.0	21.6	696	4.8	21.7	1,989	5.0	21.2	5,884
3	5.3	22.8	5.0	22.9	656	4.8	23.1	1,868	5.1	22.9	5,570
4	5.2	23.8	5.2	24.2	645	4.9	24.1	1,828	5.2	24.1	5,388
5	5.1	24.5	5.0	25.1	599	4.8	24.4	1,767	5.1	24.8	5,144
6	5.2	25.5	5.2	25.8	605	5.0	25.6	1,757	5.0	25.6	4,876
7	5.1	26.4	5.1	27.1	566	4.9	26.6	1,660	5.1	26.6	4,793
8	5.0	27.0	5.1	27.8	551	4.8	27.1	1,595	5.0	27.2	4,601
9	5.7	28.2	5.4	29.2	554	5.2	28.6	1,638	5.1	28.5	4,467
10	5.2	29.2	5.3	30.1	529	5.1	28.7	1,600	4.9	28.8	4,259
11	5.4	30.4	5.2	31.0	503	5.2	30.0	1,560	5.2	29.7	4,384
12	5.4	31.0	5.3	31.8	500	5.3	30.8	1,547	5.1	30.4	4,198
13	5.1	32.3	5.1	32.9	465	5.0	32.1	1,403	5.0	31.3	3,997
17	5.1	34.6	5.1	35.4	433	5.0	34.7	1,297	5.1	33.1	3,850
21	5.1	37.7	4.3	37.0	349	4.2	36.0	1,051	4.5	34.8	3,234
25	4.4	36.6	4.6	37.5	368	4.3	36.5	1,059	4.5	34.9	3,219
29	5.1	38.5	5.0	41.1	365	4.9	39.7	1,111	5.0	38.0	3,289
33	4.9	40.6	5.0	42.4	354	4.8	41.1	1,052	5.0	39.4	3,173
37	5.3	43.9	5.2	44.9	347	5.2	43.9	1,066	5.3	42.1	3,146
41	5.4	44.3	5.5	45.5	363	5.2	44.3	1,056	5.3	42.6	3,108
45	5.2	47.3	5.2	47.9	326	5.0	47.0	957	5.3	45.6	2,907
49	5.1	48.2	5.3	48.7	327	5.1	47.9	958	5.3	46.7	2,836
53	5.4	47.8	5.4	48.3	336	5.4	48.2	1,009	5.5	47.0	2,928
57	4.9	48.1	5.1	48.8	314	4.9	48.5	909	4.9	47.2	2,596
61	5.1	47.3	5.2	48.2	323	5.0	47.6	945	5.1	46.7	2,732
65	5.0	48.3	5.2	49.2	317	5.1	48.2	952	5.2	46.9	2,770
69	4.9	47.8	4.8	49.0	294	4.7	47.6	890	4.8	46.8	2,565
73	5.1	47.7	5.4	48.6	333	5.4	47.7	1,020	5.4	46.4	2,910
77	5.2	47.7	5.2	48.2	323	5.1	46.9	980	5.2	45.8	2,838
81	5.5	47.4	5.3	47.8	332	5.2	47.2	991	5.3	46.0	2,880
85	5.2	46.9	5.2	48.1	324	5.2	47.1	993	5.2	45.7	2,847
89	5.3	46.4	5.0	47.8	314	5.3	46.0	1,036	5.2	45.0	2,889
93	5.3	46.3	5.4	47.0	345	5.4	46.3	1,050	5.4	43.9	3,075
97	5.4	45.8	5.4	45.9	353	5.6	46.7	1,078	5.7	44.0	3,240
101	5.3	46.6	5.1	45.6	336	5.2	46.3	1,011	5.3	44.1	3,006
Mean for weeks											
1-13	5.4	26.4	5.3	26.9	609	5.1	26.4	1,789	5.3	26.3	5,129
14-52	5.1	41.3	5.0	42.3	359	4.9	41.2	1,067	5.0	39.7	3,196
53-101	5.2	47.2	5.2	47.9	326	5.2	47.3	990	5.2	45.8	2,867

[a] Grams of feed consumed per animal per day
[b] Milligrams of goldenseal root powder consumed per kilogram body weight per day

TABLE J4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Goldenseal Root Powder

Week	0 ppm		3,000 ppm			9,000 ppm			25,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ (mg/kg)
1	5.1	15.4	5.2	15.4	1,015	4.9	15.4	2,856	4.8	15.2	7,877
2	5.0	18.5	4.4	18.5	713	3.9	18.0	1,945	3.7	18.3	5,044
3	3.7	19.1	4.0	19.2	626	3.9	19.1	1,839	3.8	19.0	4,991
4	3.9	20.0	4.0	20.5	585	3.9	20.4	1,719	3.7	20.2	4,574
5	4.2	21.6	4.0	21.7	553	4.0	21.3	1,688	3.8	21.3	4,461
6	4.1	21.5	4.3	22.2	582	4.3	21.7	1,786	4.1	21.4	4,793
7	4.4	23.2	4.2	23.4	538	4.3	23.3	1,661	4.0	22.7	4,406
8	4.4	24.0	4.3	24.0	538	4.5	24.1	1,681	4.1	22.9	4,471
9	4.4	25.0	4.4	25.1	525	4.5	24.9	1,623	4.1	24.2	4,238
10	4.3	24.3	4.3	24.8	521	4.4	24.1	1,643	4.4	23.2	4,748
11	4.4	26.4	4.2	26.4	477	4.3	26.1	1,484	4.2	25.4	4,128
12	4.5	27.3	4.7	27.0	522	4.7	26.7	1,583	4.1	25.6	4,001
13	4.6	28.0	4.7	28.1	502	4.8	27.9	1,546	4.3	26.6	4,036
17	4.6	31.0	4.5	31.0	435	4.6	30.1	1,374	4.5	29.0	3,884
21	4.5	33.0	4.0	32.9	365	4.1	32.5	1,137	3.9	30.1	3,239
25	4.0	31.9	4.2	33.0	381	4.0	32.1	1,123	3.9	30.3	3,216
29	4.4	33.7	4.3	36.4	354	4.3	34.8	1,113	4.0	32.8	3,050
33	4.7	35.7	4.5	38.3	353	4.7	36.6	1,156	4.3	33.9	3,172
37	4.8	38.4	4.5	39.9	339	4.5	38.6	1,049	4.4	35.9	3,063
41	4.6	41.0	4.5	42.6	317	4.6	41.5	997	4.4	38.8	2,838
45	4.4	44.7	4.3	46.0	280	4.3	45.1	857	4.3	41.5	2,590
49	4.2	47.1	4.0	47.9	251	4.0	47.5	758	4.3	44.3	2,428
53	4.3	47.0	4.3	48.7	265	4.3	48.2	802	4.3	44.8	2,399
57	4.1	47.2	4.0	50.0	240	3.9	48.7	720	4.0	46.0	2,175
61	4.0	47.4	4.2	49.4	255	4.0	49.0	734	4.1	45.9	2,231
69	4.4	50.0	4.1	51.3	240	4.3	51.1	757	4.0	46.3	2,158
73	4.5	50.1	4.5	52.1	259	4.7	51.4	823	4.3	47.0	2,286
77	4.2	50.4	4.3	52.5	246	4.1	52.1	708	4.1	47.7	2,151
81	4.4	50.7	4.5	52.5	257	4.4	52.2	759	4.4	47.6	2,309
85	4.6	50.8	4.3	52.8	245	4.9	52.9	834	4.4	48.5	2,267
89	4.9	50.7	4.7	53.6	263	4.7	53.3	794	4.2	48.3	2,172
93	4.6	51.8	4.7	54.2	260	4.5	52.7	768	4.7	48.8	2,408
97	5.1	51.3	5.1	53.9	284	5.1	53.2	863	4.6	48.3	2,380
101	4.6	51.9	4.4	54.4	243	4.7	53.7	788	4.6	49.2	2,338
Mean for weeks											
1-13	4.4	22.6	4.4	22.8	592	4.3	22.5	1,773	4.1	22.0	4,751
14-52	4.5	37.4	4.3	38.7	342	4.3	37.6	1,063	4.2	35.2	3,053
53-101	4.5	49.9	4.4	52.1	255	4.5	51.5	779	4.3	47.4	2,273

[a] Grams of feed consumed per animal per day
[b] Milligrams of goldenseal root powder consumed per kilogram body weight per day

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

TABLE K1 Ingredients of NTP-2000 Rat and Mouse Ration 182
TABLE K2 Vitamins and Minerals in NTP-2000 Rat and Mouse Ration 182
TABLE K3 Nutrient Composition of NTP-2000 Rat and Mouse Ration 183
TABLE K4 Contaminant Levels in NTP-2000 Rat and Mouse Ration 184

TABLE K1
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 K2
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 K3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	15.0 ± 0.66	13.8 – 16.2	23
Crude fat (% by weight)	8.1 ± 0.37	7.5 – 9.0	23
Crude fiber (% by weight)	9.2 ± 0.68	8.1 – 10.6	23
Ash (% by weight)	5.0 ± 0.26	4.5 – 5.5	23
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 ± 0.033	0.21 – 0.35	18
Vitamins			
Vitamin A (IU/kg)	558 ± 332	289 – 1,760	23
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	80.6 ± 17.74	52.0 – 110.00	18
Thiamine (ppm)[b]	10.5 ± 5.89	6.4 – 30.60	23
Riboflavin (ppm)	7.1 ± 2.07	4.20 – 11.20	18
Niacin (ppm)	79.0 ± 9.79	66.4 – 98.2	18
Pantothenic acid (ppm)	27.2 ± 13.85	17.4 – 81.0	18
Pyridoxine (ppm)[b]	9.33 ± 2.13	6.4 – 13.7	18
Folic acid (ppm)	1.695 ± 0.51	1.15 – 3.27	18
Biotin (ppm)	0.321 ± 0.11	0.200 – 0.704	18
Vitamin B_{12} (ppb)	56.0 ± 43.4	18.3 – 174.0	18
Choline (ppm)[b]	3,041 ± 255	2,700 – 3,790	18
Minerals			
Calcium (%)	0.981 ± 0.054	0.906 – 1.080	23
Phosphorus (%)	0.597 ± 0.040	0.539 – 0.721	23
Potassium (%)	0.666 ± 0.028	0.626 – 0.732	18
Chloride (%)	0.384 ± 0.041	0.300 – 0.474	18
Sodium (%)	0.191 ± 0.016	0.160 – 0.222	18
Magnesium (%)	0.218 ± 0.068	0.185 – 0.490	18
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	18
Iron (ppm)	184 ± 42.7	135 – 311	18
Manganese (ppm)	51.6 ± 10.73	21.0 – 73.1	18
Zinc (ppm)	53.8 ± 9.21	43.3 – 78.5	18
Copper (ppm)	6.57 ± 1.647	3.21 – 10.50	18
Iodine (ppm)	0.524 ± 0.205	0.233 – 0.972	18
Chromium (ppm)	0.679 ± 0.295	0.330 – 1.380	17
Cobalt (ppm)	0.28 ± 0.173	0.13 – 0.86	16

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

TABLE K4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.28 ± 0.139	0.14 – 0.50	23
Cadmium (ppm)	0.07 ± 0.021	0.04 – 0.11	23
Lead (ppm)	0.09 ± 0.032	0.05 – 0.21	23
Mercury (ppm)	<0.02		23
Selenium (ppm)	0.21 ± 0.062	0.14 – 0.40	23
Aflatoxins (ppb)	<5.00		23
Nitrate nitrogen (ppm)[c]	14.9 ± 5.33	7.32 – 27.3	23
Nitrite nitrogen (ppm)[c]	<0.70		23
BHA (ppm)[d]	<1.0		23
BHT (ppm)[d]	<1.0		23
Aerobic plate count (CFU/g)	10 ± 0	10 – 10	23
Coliform (MPN/g)	3.0 ± 0.1	3.0 – 3.0	23
Escherichia coli (MPN/g)	<10		23
Salmonella (MPN/g)	Negative		23
Total nitrosoamines (ppb)[e]	4.6 ± 2.29	2.4 – 12.0	23
N-Nitrosodimethylamine (ppb)[e]	2.7 ± 1.81	1.2 – 9.3	23
N-Nitrosopyrrolidine (ppb)[e]	1.9 ± 0.78	1.0 – 3.9	23
Pesticides (ppm)			
α-BHC	<0.01		23
β-BHC	<0.02		23
γ-BHC	<0.01		23
δ-BHC	<0.01		23
Heptachlor	<0.01		23
Aldrin	<0.01		23
Heptachlor epoxide	<0.01		23
DDE	<0.01		23
DDD	<0.01		23
DDT	<0.01		23
HCB	<0.01		23
Mirex	<0.01		23
Methoxychlor	<0.05		23
Dieldrin	<0.01		23
Endrin	<0.01		23
Telodrin	<0.01		23
Chlordane	<0.05		23
Toxaphene	<0.10		23
Estimated PCBs	<0.20		23
Ronnel	<0.01		23
Ethion	<0.02		23
Trithion	<0.05		23
Diazinon	<0.10		23
Methyl chlorpyrifos	0.127 ± 0.150	0.020 – 0.450	23
Methyl parathion	<0.02		23
Ethyl parathion	<0.02		23
Malathion	0.149 ± 0.174	0.020 – 0.596	23
Endosulfan I	<0.01		23
Endosulfan II	<0.01		23
Endosulfan sulfate	<0.03		23

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

APPENDIX L
SENTINEL ANIMAL PROGRAM

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SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from five male and five female core study rats and mice in the untreated groups at termination of the 3-month studies. Serum samples were collected from five male and five female sentinel rats and mice at 6, 12, and 18 months, and from five male and five female rats and mice in the 25,000 ppm groups at the end of the 2-year studies. 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 serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed. Fecal samples were taken from ten sentinel mice at 18 months in the 2-year study.

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

Method and Test	Time of Analysis
MICE	
3-Month Study	
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
Mouse adenoma virus-FL	Study termination
MHV (mouse hepatitis virus)	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Ectromelia virus	Study termination
EDIM	Study termination
LCM	Study termination
Sendai	Study termination
Parvovirus	Study termination
Reovirus 3	Study termination
2-Year Study	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	6, 12, and 18 months, study termination
GDVII	6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
Mouse adenoma virus-FL	6, 12, and 18 months, study termination
MHV	6, 12, and 18 months, study termination
MMV	18 months, study termination
MPV	18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
Ectromelia virus	Study termination
GDVII	12 months, study termination
Mouse adenoma virus-FL	Study termination
MCMV (mouse cytomegalovirus)	Study termination
M. arthritidis	Study termination
Parvovirus	6 and 12 months
Reovirus 3	12 months, study termination
Sendai	12 months

Method and Test	Time of Analysis
Western Blot	
Reovirus 3	Study termination
Polymerase Chain Reaction	
Heliobacter species	18 months

RESULTS

All test results were negative.