NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF EMODIN

(CAS NO. 518-82-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

June 2001

NTP TR 493

NIH Publication No. 01-3952

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

FOREWORD

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

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

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

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

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Technical Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Information Service (EHIS) http://ehis.niehs.nih.gov (800-315-3010 or 919-541-3841). In addition, printed copies of these reports are available from EHIS as supplies last. A listing of all the NTP reports printed since 1982 appears on the inside back cover.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF EMODIN

(CAS NO. 518-82-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

June 2001

NTP TR 493

NIH Publication No. 01-3952

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

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

R.D. Irwin, Ph.D., Study Scientist
J.R. Bucher, Ph.D.
R.E. Chapin, Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
R.A. Herbert, D.V.M., Ph.D.
R.R. Maronpot, D.V.M.
A. Nyska, D.V.M.
D.P. Orzech, M.S.
G.N. Rao, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
K.L. Witt, M.S., Integrated Laboratory Systems, Inc.

Southern Research Institute
Conducted studies, evaluated pathology findings

J.D. Prejean, Ph.D., Principal Investigator
D.R. Farnell, D.V.M., Ph.D.
J.E. Heath, D.V.M.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
C.C. Shackelford, D.V.M., M.S., Ph.D.

Dynamac Corporation
Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

Analytical Sciences, Inc.
Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
D.E. Kendrick, M.S.
K.P. McGowan, M.B.A.
J.T. Scott, M.S.

NTP Pathology Working Group
Evaluated slides, prepared pathology report on rats (15 October 1998)

M.P. Jokinen, D.V.M., Chairperson
Pathology Associates International
J.R. Hailey, D.V.M.
National Toxicology Program
R. Miller, D.V.M., Ph.D.
North Carolina State University
A. Nyska, D.V.M.
National Toxicology Program
C.C. Shackelford, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program

Evaluated slides, prepared pathology report on mice (10 September 1998)

M. Butt, D.V.M., Chairperson
Pathology Associates International
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J. Mahler, D.V.M.
National Toxicology Program
R. Miller, D.V.M., Ph.D.
North Carolina State University
A. Nyska, D.V.M.
National Toxicology Program
C.C. Shackelford, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.

Biotechnical Services, Inc.
Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
J.A. Gregan, M.A.
L.M. Harper, B.S.
D.C. Serbus, Ph.D.
R.A. Willis, B.A., B.S.
P.A. Yount, B.S.

CONTENTS

ABSTRACT 5

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY 10

TECHNICAL REPORTS REVIEW SUBCOMMITTEE 11

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS 12

INTRODUCTION 15

MATERIALS AND METHODS 19

RESULTS 31

DISCUSSION AND CONCLUSIONS 59

REFERENCES 63

APPENDIX A Summary of Lesions in Male Rats in the 2-Year Feed Study of Emodin 69

APPENDIX B Summary of Lesions in Female Rats in the 2-Year Feed Study of Emodin 103

APPENDIX C Summary of Lesions in Male Mice in the 2-Year Feed Study of Emodin 133

APPENDIX D Summary of Lesions in Female Mice in the 2-Year Feed Study of Emodin 169

APPENDIX E Genetic Toxicology 207

APPENDIX F Clinical Pathology Results 217

APPENDIX G Organ Weights and Organ-Weight-to-Body-Weight Ratios 223

APPENDIX H Reproductive Tissue Evaluations and Estrous Cycle Characterization 231

APPENDIX I Determinations of Emodin in Plasma 235

APPENDIX J Chemical Characterization and Dose Formulation Studies 239

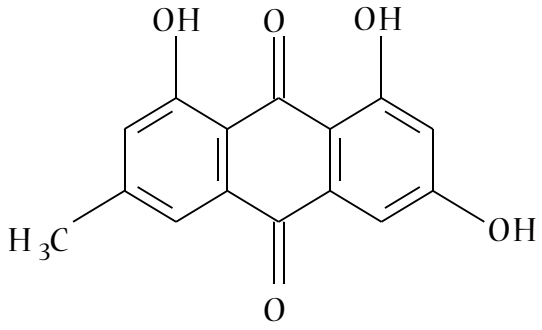
APPENDIX K Feed and Compound Consumption in the 2-Year Feed Studies of Emodin 257

APPENDIX L Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration 263

APPENDIX M Sentinel Animal Program 267

APPENDIX N Single-Dose Toxicokinetic Studies in F344/N Rats and B6C3F$_1$ Mice 271

ABSTRACT



EMODIN

CAS No. 518-82-1

Chemical Formula: $C_{15}H_{10}O_5$ Molecular Weight: 270.23

Synonyms: Archin; C.I. 75440; C.I. Natural Green 2; C.I. Natural Yellow 14; emodol; frangulic acid; frangula emodin; 6-methyl-1,3,8-trihydroxyanthraquinone; Persian Berry Lake; rheum emodin; schuttgelb; 1,3,8-trihydroxy-6-methyl-9,10-anthracenedione; 1,3,8-trihydroxy-6-methylanthraquinone; 4,5,7-trihydroxy-2-methylanthraquinone

Emodin is a naturally occurring anthraquinone present in the roots and bark of numerous plants of the genus *Rhamnus*. Extracts from the roots, bark, and/or dried leaves of buckthorn, senna, cascara, aloe, frangula, and rhubarb have been used as laxatives since ancient times and currently are widely used in the preparation of herbal laxative preparations. Anthraquinone glycosides are poorly absorbed from the gastrointestinal tract but are cleaved by gut bacteria to produce aglycones (such as emodin) that are more readily absorbed and are responsible for the purgative properties of these preparations. There is extensive exposure to emodin and other anthraquinones resulting from the use of herb-based stimulant laxatives. Reports that 1,8-dihydroxyanthraquinone, a commonly used laxative ingredient, caused tumors in the gastrointestinal tract of rats raised the possibility of an association between colorectal cancer and the use of laxatives containing anthraquinones. Because emodin is a hydroxyanthraquinone structurally similar to 1,8-dihydroxyanthraquinone, is present in herbal laxatives, and was reported to be mutagenic in bacteria, it was considered a potential carcinogen and was selected for in-depth evaluation. Male and female F344/N rats and B6C3F$_1$ mice were exposed to emodin (at least 94% pure) in feed for 16 days, 14 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, rat and mouse bone marrow cells, and mouse peripheral blood erythrocytes.

16-Day Study in Rats

Groups of five male and five female rats were fed diets containing 0, 600, 2,000, 5,500, 17,000, or 50,000 ppm emodin (equivalent to average daily doses of approximately 50, 170, 480, 1,400, or 3,700 mg emodin/kg body weight to males and 50, 160, 460, 1,250, or 2,000 mg/kg to females) for 15 (males) or 16 (females) days. Three female rats died before the end of the study. Mean body weights of males and females exposed to 5,500 ppm or greater were significantly less than those of the controls. Feed

consumption by males and females receiving 17,000 or 50,000 ppm was decreased throughout the study. Macroscopic lesions were present in the kidney of rats exposed to 17,000 or 50,000 ppm.

16-Day Study in Mice

Groups of five male and five female mice were fed diets containing 0, 600, 2,000, 5,500, 17,000, or 50,000 ppm emodin (equivalent to average daily doses of approximately 120, 400, 1,200, or 3,800 mg/kg to males and 140, 530, 1,600, or 5,000 mg/kg to females; 50,000 ppm equivalents were not calculated due to high mortality) for 15 (males) or 16 (females) days. All mice exposed to 50,000 ppm died before the end of the study. Mice in the 17,000 ppm groups lost weight during the study. Feed consumption by 5,500 ppm females was greater than that by the controls throughout the study. Macroscopic lesions were present in the gallbladder and kidney of mice exposed to 17,000 ppm.

14-Week Study in Rats

Groups of 10 male and 10 female rats were fed diets containing 0, 312.5, 625, 1,250, 2,500, or 5,000 ppm emodin (equivalent to average daily doses of approximately 20, 40, 80, 170, or 300 mg/kg to males and females) for 14 weeks. Mean body weights of males exposed to 2,500 ppm or greater and females exposed to 1,250 ppm or greater were significantly less than those of the controls. During the first week of the study, feed consumption by males exposed to 2,500 or 5,000 ppm and females exposed to 5,000 ppm was less than that by the controls. Feed consumption by these groups was similar to that by the controls for the remainder of the study. In rats exposed to 2,500 or 5,000 ppm, there were increases in platelet counts in males and females and segmented neutrophil counts in females. Total serum protein and albumin concentrations were decreased in females exposed to 2,500 or 5,000 ppm. Relative kidney weights of rats exposed to 1,250 ppm or greater and relative lung weights of rats exposed to 625 ppm or greater were significantly increased compared to the control groups. Relative liver weights were increased in females exposed to 625 ppm or greater. The estrous cycle length was significantly increased in females exposed to 1,250 or 5,000 ppm.

All male rats exposed to 1,250 ppm or greater and all exposed female rats had pigment in the renal tubules; and the severity of pigmentation generally increased with increasing exposure concentration. The incidences of hyaline droplets in the cortical epithelial cytoplasm were increased in all groups of exposed males and in females exposed to 312.5, 625, or 1,250 ppm.

14-Week Study in Mice

Groups of 10 male and 10 female mice were fed diets containing 0, 312.5, 625, 1,250, 2,500, or 5,000 ppm emodin (equivalent to average daily doses of approximately 50, 100, 190, 400, or 800 mg/kg to males and 60, 130, 240, 500, or 1,100 mg/kg to females) for 14 weeks. All mice survived to the end of the study. Mean body weights of males exposed to 2,500 or 5,000 ppm were significantly less than those of the controls. Feed consumption by exposed groups was generally similar to that by the controls. Relative kidney weights of male mice exposed to 1,250 ppm or greater, relative lung weights of males exposed to 625 ppm or greater, and relative liver weights of female mice exposed to 625 ppm or greater were increased.

The incidences and severities of nephropathy were increased in males and females exposed to 1,250 ppm or greater. The incidences of renal tubule pigmentation were significantly increased in males exposed to 625 ppm or greater and in females exposed to 1,250 ppm or greater.

2-Year Study in Rats

Groups of 65 male and 65 female rats were fed diets containing 0, 280, 830, or 2,500 ppm emodin (equivalent to average daily doses of approximately 110, 320, or 1,000 mg/kg to males and 120, 370, or 1,100 mg/kg to females) for 105 weeks. Ten male and ten female rats from each group were necropsied at 6 months. Blood samples from five male and five female rats in each group were evaluated at 3, 6, and 12 months for plasma emodin concentrations; these rats were necropsied at 12 months.

Survival, Body Weights, and Feed Consumption

Survival of exposed males and females was similar to that of the controls. The mean body weights of rats in the 2,500 ppm groups were less than those of the controls beginning at week 2 of the study. Feed consumption by exposed groups was similar to that by the controls throughout the study.

Pathology Findings

Three Zymbal's gland carcinomas were observed in female rats exposed to 2,500 ppm. This incidence exceeded the range observed for current historical controls and was considered an equivocal finding.

At the 6- and 12-month interim evaluations and at 2 years, emodin-related increases in the incidences of renal tubule hyaline droplets occurred in all exposed groups. The incidences of renal tubule pigmentation were significantly increased in all exposed groups of males at 2 years.

There were negative trends in the incidences of mononuclear cell leukemia in male and female rats, and the incidences in the 2,500 ppm groups were significantly decreased. In females exposed to 2,500 ppm, the incidence was below the historical control range; the incidence in males exposed to 2,500 ppm was at the lower end of the historical control range.

2-Year Study in Mice

Groups of 60 male mice were fed diets containing 0, 160, 312, or 625 ppm emodin (equivalent to average daily doses of approximately 15, 35, or 70 mg/kg) for 105 weeks. Groups of 60 female mice were fed diets containing 0, 312, 625, or 1,250 ppm emodin (equivalent to average daily doses of approximately 30, 60, or 120 mg/kg) for 105 weeks. Ten male and ten female mice from each group were necropsied at 12 months.

Survival, Body Weights, and Feed Consumption

Survival and mean body weights of exposed males and females were similar to those of the controls. No differences in feed consumption were noted between exposed and control groups.

Pathology Findings

Low incidences of renal tubule adenoma and carcinoma occurred in exposed male mice; these incidences included one carcinoma each in the 312 and 625 ppm groups. Renal tubule neoplasms are rare in male mice, and their presence in these groups suggested a possible association with emodin exposure.

At the 12-month interim evaluation, the severity of nephropathy was slightly increased in males exposed to 625 ppm. Also at 12 months, the severity of nephropathy increased from minimal in the lower exposure groups to mild in females exposed to 1,250 ppm; the incidence in this group was significantly increased compared to the control group. At 2 years, the severities of nephropathy were slightly increased in males exposed to 625 ppm and females exposed to 1,250 ppm. The incidences of nephropathy were significantly increased in all exposed groups of females. At the 12-month interim evaluation, the incidences of renal tubule pigmentation were significantly increased in all exposed groups of males and in females exposed to 625 or 1,250 ppm. The severities increased with increasing exposure concentration. At 2 years, the incidences of renal tubule pigmentation were significantly increased in all exposed groups; severities increased with increasing exposure concentration.

Genetic Toxicology

Emodin was mutagenic in *Salmonella typhimurium* strain TA100 in the presence of S9 activation; no mutagenicity was detected in strain TA98, with or without S9. Chromosomal aberrations were induced in cultured Chinese hamster ovary cells treated with emodin, with and without S9. Three separate *in vivo* micronucleus tests were performed with emodin. A male rat bone marrow micronucleus test, with emodin administered by three intraperitoneal injections, gave negative results. Results of acute-exposure (intraperitoneal injection) micronucleus tests in bone marrow and peripheral blood erythrocytes of male and female mice were negative. In a peripheral blood micronucleus test on mice from the 14-week study, negative results were seen in male mice, but a weakly positive response was observed in similarly exposed females.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of emodin in male F344/N rats exposed to 280, 830, or 2,500 ppm. There was *equivocal evidence of carcinogenic activity* of emodin in female F344/N rats based on a marginal increase in the incidence of Zymbal's gland carcinoma. There was *equivocal evidence of carcinogenic activity* of emodin in male $B6C3F_1$ mice based on a low incidence of uncommon renal tubule neoplasms. There was *no evidence of carcinogenic activity* of emodin in female $B6C3F_1$ mice exposed to 312, 625, or 1,250 ppm.

Exposure of rats to emodin resulted in increased incidences of renal tubule hyaline droplets and pigmentation in males, increased incidences of renal tubule hyaline droplets in females, and increased severities of renal tubule pigmentation in males and females. Emodin exposure resulted in increased incidences of renal tubule pigmentation in male and female mice and increased incidences of nephropathy in female mice.

Incidences of mononuclear cell leukemia decreased in male and female rats exposed to 2,500 ppm.

---

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

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

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in feed	0, 280, 830, or 2,500 ppm	0, 280, 830, or 2,500 ppm	0, 160, 312, or 625 ppm	0, 312, 625, or 1,250 ppm
Body weights	2,500 ppm group less than control group	2,500 ppm group less than control group	Exposed groups similar to control group	Exposed groups similar to control group
Survival rates	30/50, 21/50, 21/50, 30/50	33/50, 39/50, 35/50, 34/50	41/50, 37/50, 40/50, 43/50	37/50, 39/50, 40/50, 36/50
Nonneoplastic effects	Kidney: renal tubule hyaline droplet (3/50, 45/50, 43/50, 43/50); renal tubule pigmentation (35/50, 47/50, 49/50, 50/50); severity of pigmentation (1.3, 1.8, 1.8, 2.1)	Kidney: renal tubule hyaline droplet (22/49, 49/50, 49/49, 50/50); severity of pigmentation (1.2, 1.4, 2.4, 3.0)	Kidney: renal tubule pigmentation (0/49, 46/50, 50/50, 50/50)	Kidney: renal tubule pigmentation (0/49, 37/50, 48/50, 49/49); nephropathy (22/49, 46/50, 41/50, 48/49)
Neoplastic effects	None	None	None	None
Uncertain findings	None	Zymbal's gland: carcinoma (0/50, 0/50, 0/50, 3/50)	Kidney: renal tubule adenoma (standard evaluation - 0/49, 1/50, 1/50, 0/50; standard and extended evaluations combined - 0/49, 1/50, 1/50, 1/50); renal tubule carcinoma (standard evaluation - 0/49, 0/50, 1/50, 1/50; renal tubule adenoma or carcinoma (standard evaluation - 0/49, 1/50, 2/50, 1/50; standard and extended evaluations combined - 0/49, 1/50, 2/50, 2/50)	None
Decreased incidences	Mononuclear cell leukemia: 28/50, 31/50, 29/50, 18/50	Mononuclear cell leukemia: 14/50, 17/50, 16/50, 3/50	None	None
Level of evidence of carcinogenic activity	No evidence	Equivocal	Equivocal	No evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Positive in strain TA100 with S9; negative in strain TA100 without S9; negative with and without S9 in strain TA98			
Chromosomal aberrations				
Cultured Chinese hamster ovary cells *in vitro*:	Positive with and without S9			
Micronucleated erythrocytes				
Rat bone marrow *in vivo*:	Negative when administered as intraperitoneal injections			
Mouse bone marrow *in vivo*:	Negative when administered as intraperitoneal injections			
Mouse peripheral blood *in vivo*:	Negative when administered as intraperitoneal injections; negative in males and weakly positive in females when administered in feed for 14 weeks			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on emodin on 21 May 1999 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.

Gary P. Carlson, Ph.D., Chairperson
School of Health Sciences
Purdue University
West Lafayette, IN

A. John Bailer, Ph.D.
Department of Mathematics and Statistics
Miami University
Oxford, OH

Steven A. Belinsky, Ph.D.
Inhalation Toxicology Research Institute
Kirkland Air Force Base
Albuquerque, NM

James S. Bus, Ph.D.*
Health and Environmental Sciences
Dow Chemical Company
Midland, MI

Linda A. Chatman, D.V.M., Principal Reviewer
Pfizer, Inc.
Groton, CT

John M. Cullen, Ph.D., V.M.D.
Department of Microbiology, Parasitology, and Pathology
College of Veterinary Medicine
North Carolina State University
Raleigh, NC

Harold Davis, D.V.M., Ph.D.
Director of Toxicology
Amgen, Inc.
Thousand Oaks, CA

Susan M. Fischer, Ph.D.
M.D. Anderson Cancer Center
The University of Texas
Smithville, TX

Stephen S. Hecht, Ph.D.
University of Minnesota Cancer Centers
Minneapolis, MN

Michele Medinsky, Ph.D., Principal Reviewer
Durham, NC

Jose Russo, M.D., Principal Reviewer
Fox Chase Cancer Center
Philadelphia, PA

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 21 May 1999, the draft Technical Report on the toxicology and carcinogenesis studies of emodin received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

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

Dr. Hecht, a principal reviewer, agreed with the proposed conclusions, but he thought that squamous cell carcinomas of the nose in rats should be considered related to emodin exposure. Because the rat nose, known to be a rich source of cytochrome P450 enzymes, could metabolically activate emodin, he said the possibility of a relationship between the neoplasms to emodin exposure could not be categorically ruled out. Dr. Irwin agreed that the potential for metabolic activation existed, but there were just two animals with nasal neoplasms and there were no other indications of preneoplastic activity such as squamous metaplasia.

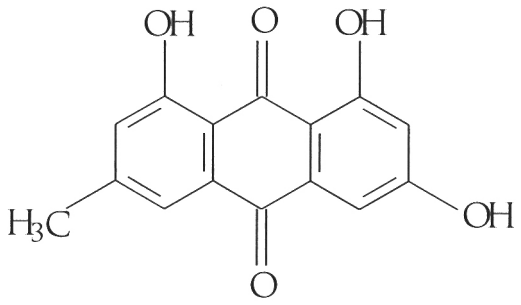
Dr. Chatman, the second principal reviewer, agreed with the proposed conclusions. She asked why emodin, a cathartic, did not show laxative effects in the 2-year studies and whether water consumption was monitored. Dr. Irwin said the lack of cathartic effects was a surprise and that water consumption was not specifically monitored. In view of conflicting results on genotoxicity, Dr. Chatman asked if there were any additional studies planned. She noted the first pass effect and need for metabolic activation suggesting a metabolite as the genotoxic form. Dr. Irwin responded that further studies were not planned and that 2-hydroxyemodin, a metabolite, acts as the genotoxin. Dr. Chatman noted increased estrogenic activity of emodin reported in an early study and asked if there was a potential for reproductive problems in women who abuse laxatives. Dr. Irwin said endocrine disruptor screening of emodin would be worthwhile.

Dr. Russo, the third principal reviewer, agreed with the proposed conclusions and asked about estrogenic effects of emodin. Dr. Irwin reported a lengthening of the estrous cycle in exposed rats but no changes in morphology in the uterus.

There was considerable discussion as to whether increased incidences of bone marrow hyperplasia and hematopoietic cell proliferation in rats were exposure-related effects or secondary to decreased incidences of mononuclear cell leukemia in male and female rats. Drs. J.R. Hailey and A. Nyska, NIEHS, concluded that these increases were secondary to decreased mononuclear cell leukemia and agreed to add the interpretation in the report. Dr. Medinsky suggested more discussion on the toxicokinetics, noting that low concentrations of emodin in the blood could be due to poor gastrointestinal absorption and/or an extensive first pass effect by the liver in which a majority of the parent compound is metabolized. Dr. Irwin agreed and noted that extensive studies were not done due to limited amounts of the test material and because there was considerable information on metabolism and disposition in the literature. Dr. Cullen observed that emodin may not be a cathartic in rodents due to a different gut structure from humans and certainly different reabsorption capability in colonic function. Dr. Bailer questioned the lack of attention to several small, apparently exposure concentration related increases in neoplasms, such as Harderian gland carcinomas. Dr. Hailey explained that NTP considers analysis of benign and malignant neoplasms combined to be the most relevant when looking at tumorigenic effects, and in doing this with the Harderian gland, there was neither a positive trend nor pairwise differences. Dr. Chatman asked if possible antileukemic effects of emodin were being evaluated. Dr. Irwin replied that emodin, as well as some derivatives, was being evaluated for human use as an anticancer agent.

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

INTRODUCTION



EMODIN

CAS No. 518-82-1

Chemical Formula: $C_{15}H_{10}O_5$ Molecular Weight: 270.23

Synonyms: Archin; C.I. 75440; C.I. Natural Green 2; C.I. Natural Yellow 14; emodol; frangulic acid; frangula emodin; 6-methyl-1,3,8-trihydroxyanthraquinone; Persian Berry Lake; rheum emodin; schuttgelb; 1,3,8-trihydroxy-6-methyl-9,10-anthracenedione; 1,3,8-trihydroxy-6-methylanthraquinone; 4,5,7-trihydroxy-2-methylanthraquinone

CHEMICAL AND PHYSICAL PROPERTIES

Emodin is a fluffy, orange powder that is insoluble in water but slightly soluble in ether, chloroform, carbon tetrachloride, carbon bisulfide, and benzene, and soluble in alcohol. The melting point ranges from 256° to 257° C (*Merck Index*, 1989; Lide, 1998).

PRODUCTION, USE, AND HUMAN EXPOSURE

Emodin is a naturally occurring anthraquinone present in the roots and bark of numerous plants of the genus *Rhamnus*. Emodin occurs primarily as a mixture of two glycosides: the 3-O-glycoside of L-rhamnose (frangulin A) and the 3-O-glycoside of D-apiofuranose (frangulin B). Extracts from the roots, bark, and/or dried leaves of buckthorn, senna, cascara, aloe, frangula, and rhubarb have been used as laxatives since ancient times and currently are widely used in the preparation of herbal laxative preparations. Anthraquinone glycosides are poorly absorbed from the gastrointestinal tract but are cleaved by gut bacteria to produce aglycones (such as emodin) that are more readily absorbed and are responsible for the purgative properties of these preparations (Brunton, 1985). Herbal laxatives are nonprescription products that are readily available to the public. Production figures for herbal laxatives are not available.

Free, unconjugated emodin is also a mycotoxin formed by several strains of fungi, as well as a component of several *bis*-anthraquinone mycotoxins (Ueno, 1984). Emodin can also be synthesized in a Friedel-Crafts reaction from 3,5-dinitrophthalic anhydride and *m*-cresol.

Extensive exposure to emodin and other anthraquinones results from the use of herb-based stimulant laxatives. Approximately 15% to 20% of the American population suffers from constipation and uses some type of laxative on a regular basis (Everhart

et al., 1989). In addition, because stimulant laxatives are effective and readily available, these products are widely abused by people with eating disorders such as anorexia and bulimia. Melanosis coli, a brown discoloration of the colonic mucosa caused by the presence of pigment-laden macrophages, is an indicator of long-term use of laxatives containing anthraquinone. In a recent retrospective study of a cohort of 2,277 colonoscopy patients, melanosis coli was observed in 4.3% of all the cases, whereas melanosis coli was present in 14.7% of patients suffering from constipation (Nusko *et al.*, 1993). Previous studies of a proctologic examination series reported an overall melanosis coli prevalence of 1% to 8% with a prevalence of 12% to 31% in patients suffering from constipation (Nusko *et al.*, 1993). With the withdrawal of phenolphthalein-based laxatives from the market and current trends favoring the use of remedies based on natural products, it is likely that the use of anthraquinone-containing laxatives will increase.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

The disposition and metabolism of emodin has been examined in rats by Bachmann and Schlatter (1981). Oral administration of ^{14}C-emodin (50 mg/kg) revealed rapid absorption from the gastrointestinal tract. Radioactivity in the peripheral blood reached a maximum 2 hours after administration and had decreased to 30% of the peak value within 24 hours; however, detectable quantities were present in the peripheral blood 5 days later. Radioactivity appeared in urine 4 to 6 hours after administration, and total cumulative radioactivity recovered in urine amounted to 18% of the administered dose after 24 hours and 22% after 120 hours. In the urine, 70% of the radioactivity was in the form of free anthraquinone, and the remainder was present as glucuronide or sulfate conjugates. Cumulative radioactivity excreted in the feces amounted to 44% of the administered dose within 24 hours and a total of 66% after 72 hours. In the feces, 62% of the radioactivity was in the form of free anthraquinones; the remaining material was unextractable. The only metabolite identified in the urine was emodic acid (1,3,8-trihydroxy-6-carboxyanthraquinone).

In rats in which the bile duct had been cannulated, radioactivity appeared in bile within 30 minutes of administration, reached a maximum by 6 hours, and decreased rapidly thereafter so that by 15 hours after administration only traces of radioactivity were detectable in bile. Total biliary excretion amounted to 50% of the administered dose within 15 hours. In cannulated animals 7% of the administered dose was eliminated in urine and 45% in feces compared to 22% and 67%, respectively, in noncannulated animals, thus suggesting considerable enterohepatic recirculation. In cannulated animals, metabolites and parent compound excreted in bile were almost entirely in the form of conjugates whereas in noncannulated animals, parent and metabolites present in the urine were mostly in the free unconjugated form. This result is consistent with the ability of gut bacteria to efficiently cleave conjugated material entering the intestine in bile.

The biotransformation of emodin by liver microsomal preparations *in vitro* has also been examined. Masuda and Ueno (1984) incubated emodin with microsomes prepared from rats pretreated with PCB, 3-methylcholanthrene, or phenobarbital, and detected at least five anthraquinoid metabolites, only one of which was a direct-acting mutagen in bacteria. Of the five metabolites, only 2-hydroxyemodin, the mutagenic metabolite, was positively identified. Additionally, it was demonstrated that the formation of 2-hydroxyemodin was inhibited by carbon monoxide, SKF 525A, and α-naphthoflavone. In subsequent studies (Masuda *et al.*, 1985; Murakami *et al.*, 1987), four additional metabolites were identified as 4-hydroxyemodin, 5-hydroxyemodin, 7-hydroxyemodin, and ω-hydroxyemodin, in which the 6-methyl group was hydroxylated. Mueller *et al.* (1998a) incubated emodin with liver microsomes from male and female rats and detected the formation of two metabolites identified as 2-hydroxyemodin and ω-hydroxyemodin. The formation of 2-hydroxyemodin was stimulated by pretreatment with 3-methylcholanthrene and inhibited by α-naphthoflavone. 2-Hydroxyemodin formation was also inhibited by anti-rat cytochrome P4501A1/2 and to a lesser extent by anti-rat cytochrome P4501A1.

Humans

No information on the absorption, distribution, metabolism, and excretion of emodin in humans was found in a search of the available literature.

TOXICITY

Only acute toxicity data in mice are available for emodin. The intraperitoneal LD_{50} (dimethylsulfoxide solvent) is 35 mg/kg; the oral LD_{50} (dimethylsulfoxide solvent) is greater than 1,000 mg/kg (Bachmann *et al.*, 1979). No information on the toxicity of emodin in humans was found in a search of the available literature.

CARCINOGENICITY

Experimental Animals

The carcinogenic potential of emodin has not been evaluated; however, two hydroxyanthraquinones structurally similar to emodin have been evaluated for carcinogenic potential. Mori *et al.* (1985) administered diets containing 1% 1,8-dihydroxyanthraquinone to 18 ACI rats (gender unspecified) for 16 months. A second group of 15 ACI rats was given basal diet and served as the control group. A total of seven rats in the treated group developed adenomas or adenocarcinomas of the colon or cecum whereas none of the rats in the control group had tumors of the cecum or colon. In a second study, Mori *et al.* (1986) administered diets containing 0% or 0.2% 1,8-dihydroxyanthraquinone to a group of 20 C3H/HeN mice (gender unspecified) for 540 days. All 17 surviving treated mice developed adenomatous hyperplasia of the cystic glands in the cecum and five surviving mice had similar lesions in the colon; none occurred in the control group. In addition, hepatocellular carcinoma was observed in four surviving treated mice, and none occurred in the control group.

1-Hydroxyanthraquinone was administered at 1% in the diet to 30 ACI/N male rats for 480 days; there was an equal number of untreated controls (Mori *et al.*, 1990). Adenomas or adenocarcinomas of the cecum or upper portion of the colon occurred in 25 of 29 rats in the treated group and in none in the control group. In addition, 12 treated animals had neoplastic nodules or hepatocellular carcinomas and five treated rats had stomach tumors.

Several other anthraquinones containing amino or halogen moieties have been evaluated for carcinogenic potential. 2-Aminoanthraquinone was administered in feed at time-weighted average concentrations of 0%, 0.35%, or 0.69% to groups of 50 male F344/N rats, 0% or 0.2% to groups of 50 female F344/N rats, and 0%, 0.5%, or 1% to groups of 50 male and 50 female $B6C3F_1$ mice for 78 to 80 weeks. At the end of the exposure period, the animals were switched to control feed. Rats were then held an additional 32 weeks and mice an additional 16 weeks. 2-Aminoanthraquinone induced hepatocellular neoplasms in male rats and male and female mice (NCI, 1978a).

1-Amino-2-methylanthraquinone was administered in feed at time-weighted average concentrations to groups of 45 to 50 male and 45 to 50 female F344/N rats and $B6C3F_1$ mice. Rats received diets containing 0%, 0.1%, or 0.2% for 78 weeks followed by a 26- to 28-week observation period during which control feed was provided. Mice received 0% or 0.6% for 73 weeks followed by a 24- to 25-week observation period. Exposure to 1-amino-2-methylanthraquinone significantly increased incidences of hepatocellular neoplasms in male and female rats and female mice and renal neoplasms in male rats (NCI, 1978b).

2-Methyl-1-nitroanthraquinone was administered in feed to groups of 50 male and 50 female F344/N rats at concentrations of 0%, 0.06%, or 0.12% for 78 weeks followed by a 31-week observation period during which the animals were not exposed. A significant increase in the incidence of hepatocellular neoplasms in male and female rats was associated with exposure to 2-methyl-1-nitroanthraquinone (NCI, 1978c).

1,4,5,8-Tetraaminoanthraquinone (C.I. Disperse Blue 1) was administered to groups of 50 male and 50 female F344/N rats at dietary concentrations of 0, 1,250, 2,500, or 5,000 ppm and to groups of 50 male and 50 female $B6C3F_1$ mice at dietary concentrations of 0, 600, 1,200, or 2,500 ppm for 2 years. Chemical exposure was associated with significant increases in the incidences of bladder neoplasms in male and female rats and hepatocellular neoplasms and alveolar/bronchiolar neoplasms in male and female mice (NTP, 1986).

1-Amino-2,4-dibromoanthraquinone was administered in feed to groups of 50 male and 50 female F344/N rats and $B6C3F_1$ mice for 2 years. Rats received concentrations of 0, 2,000, 5,000, or 10,000 ppm and mice received 0, 10,000, or 20,000 ppm. Exposure to 1-amino-2,4-dibromoanthraquinone was associated with significant

increases in the incidences of neoplasms of the large intestine, kidney, liver, and urinary bladder in rats and of neoplasms of the forestomach, liver, and lung in mice (NTP, 1996).

Humans

No information on the carcinogenicity of emodin in humans was found in a search of the available literature.

GENETIC TOXICITY

Emodin has shown mutagenic activity in several *in vitro* assays when testing occurred in the presence of metabolic activation enzymes. Results have not always been consistent within an assay or among testing laboratories, and no activity was demonstrated in the single *in vivo* test that was reported.

No differential growth inhibition in DNA repair-deficient strains of *Bacillus subtilis* (Ueno and Kubota, 1976) or *Escherichia coli* (Fluck *et al.*, 1976) was observed after treatment with emodin, but frame-shift mutations induced by emodin at the histidine locus in *Salmonella typhimurium* strains TA1537 and/or TA1538 have been demonstrated by several laboratories (Brown and Brown, 1976; Wehner *et al.*, 1979; Cheh *et al.*, 1980; Liberman *et al.*, 1980; Tikkanen *et al.*, 1983; Bruggeman and van der Hoeven, 1984; Masuda and Ueno, 1984; Westendorf *et al.*, 1990; Krivobok *et al.*, 1992). S9 activation was required for the mutagenic response in *Salmonella* tests. A recent comparative plate incorporation mutagenicity study with emodin using two strains of *S. typhimurium* and two strains of *E. coli* that are known to respond to chemicals that produce reactive oxygen species demonstrated a two-fold increase in revertants in *S. typhimurium* strains TA102 and TA2638 in the presence of S9 (Watanabe *et al.*, 1998). Neither of the two *E. coli* WP2 derivatives were mutated by emodin in this study. Earlier *Salmonella* test data gave negative results with emodin in strain TA102, tested over comparative concentration ranges, with and without S9 (Westendorf *et al.*, 1990; Krivobok *et al.*, 1992).

Tests for induction of unscheduled DNA synthesis in rat or mouse primary hepatocyte cultures have given conflicting results, with Mori *et al.* (1984) reporting negative results and Westendorf *et al.* (1990) reporting positive results. Tests for induction of sister chromatid exchanges in hamster V79 cells (Bruggeman and van der Hoeven, 1984) gave negative results for emodin. Gene mutation tests with emodin at the HGPRT locus in hamster V79 cells also gave conflicting results in different laboratories, with negative results reported by Bruggeman and van der Hoeven (1984) and positive results reported by Westendorf *et al.* (1990). In addition, weakly positive results were reported with emodin, in the absence of S9, for gene mutation and micronucleus induction in L5178Y mouse lymphoma cells (Müller *et al.*, 1996). Emodin also induced micronuclei in Syrian hamster embryo cells at concentrations from 13.75 to 25 μg/mL (Gibson *et al.*, 1997); S9 was not required for activation, as these cells are metabolically competent. Emodin was also positive in the Syrian hamster embryo cell transformation assay (Kerckaert *et al.*, 1996). Mueller *et al.* (1998b) reported that emodin mutagenicity in mammalian cell cultures is directly influenced by its high serum protein binding affinity and they suggested that weak or inconsistent mutagenicity test results reported in earlier studies with emodin may have resulted from the selective scavenging of emodin by supplemental serum added to the culture medium.

Despite the positive *in vitro* micronucleus test results with emodin, results from an *in vivo* study in male and female mice showed no induction of micronuclei in bone marrow cells 24 or 48 hours after a single oral dose of 2,000 mg/kg emodin (plasma levels up to 190 μg/mL emodin were measured) (Mengs *et al.*, 1997).

STUDY RATIONALE

Reports (Mori *et al.*, 1985, 1986) that 1,8-dihydroxyanthraquinone, a commonly used laxative ingredient, caused tumors in the gastrointestinal tract of rats raised the possibility of an association between colorectal cancer and the use of laxatives containing anthraquinones. Because emodin is a hydroxyanthraquinone structurally similar to 1,8-dihydroxyanthraquinone, is present in herbal laxatives, and was reported to be mutagenic in bacteria, it was considered a potential carcinogen and was selected for in-depth evaluation.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF EMODIN

Emodin was obtained from Aldrich Chemical Company (Milwaukee, WI) in four lots (99912ET, EW11728DW, 06625DG, and 13309EG) and from Pfaltz & Bauer, Inc. (Waterbury, CT), in one lot (040221). Lot 99912ET was used during the 16-day studies, and lot EW11728DW was used during the 14-week studies. All lots were combined as lot SRI-A09/L7 and used during the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) and the study laboratory; stability analyses were conducted by the analytical chemistry laboratory (Appendix J). Reports on analyses performed in support of the emodin studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a fluffy, orange powder, was identified as emodin by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of lots 99912ET and EW11728DW was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) at the analytical chemistry laboratory. The purity of lot SRI-A09/L7 was determined by elemental analyses, Karl Fischer water analysis, gas chromatography/mass spectroscopy (GC/MS), and HPLC at the study laboratory.

For lot 99912ET, elemental analyses were in agreement with the theoretical values for emodin. Karl Fischer water analysis indicated less than 0.4% water. Functional group titration indicated a purity of 101.1% ± 0.6%. TLC by two systems indicated a major peak and two or three trace impurities. HPLC at two wavelengths indicated one major peak and four impurities with combined areas of 1.8% and 1.5%, respectively, relative to the major peak area. The cumulative data indicated a purity of approximately 98%.

For lot EW11728DW, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for emodin. Karl Fischer water analysis indicated 0.34% ± 0.03% water. Functional group titration indicated a purity of 93.7% ± 0.5%. TLC by both systems revealed one major peak and two minor impurities. HPLC at both wavelengths (254 and 436 nm) indicated one major peak and three impurities with combined areas of 7.0% and 6.2%, respectively, relative to the major peak area. Major peak comparisons of lot EW11728DW with lot 99912ET by HPLC indicated a purity of 96.4% ± 0.5% for lot EW11728DW relative to lot 99912ET. The overall purity of lot EW11728DW was determined to be approximately 94%. Lot EW11728DW was further characterized by HPLC/MS. Two impurity peaks with areas greater than 1% relative to the major peak were resolved by this system; one impurity, with a molecular weight of 254, was tentatively identified as 1,8-dihydroxy-3-methylanthraquinone, and the other, with a molecular weight of 284, as 1,8-dihydroxy-3-methoxy-6-methylanthraquinone. HPLC by was used to confirm the identity of and quantitate the impurity with the molecular weight of 254; 2.13% ± 0.2% 1,8-dihydroxy-3-methylanthraquinone was confirmed. No standard for the second impurity was commercially available.

For lot SRI-A09/L7, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for emodin. Karl Fischer water analysis indicated 0.94% ± 0.13% water. GC/MS indicated one major peak and two impurities with a combined area of 3.4% relative to the major peak area; mass spectra of these impurities were identical to those of chrysophenic acid and physcion. HPLC indicated one major peak and two impurities with a combined area of 2.94% relative to the major peak area and confirmed the impurities as chrysophanic acid and physcion. The overall purity of lot SRI-A09/L7 was approximately 96.1%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using HPLC. These studies indicated that emodin is stable for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature in sealed containers protected from light. Stability was monitored throughout the studies using HPLC. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared once for the 16-day studies and every 2 weeks for the 14-week and 2-year studies by mixing emodin with feed (Table J2). Formulations were stored in plastic bags in opaque plastic buckets at room temperature for up to 3 weeks. Homogeneity and stability studies of a 500 ppm dose formulation were performed by the analytical chemistry laboratory using HPLC. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for 3 weeks at 5° C when stored in the dark.

Periodic analyses of the dose formulations of emodin were conducted at the study laboratory using HPLC. Dose formulations were analyzed at the beginning of the 16-day studies (Table J3), at the beginning, midpoint, and end of the 14-week studies (Table J4), and approximately every 10 weeks during the 2-year studies (Table J5). All the dose formulations in the 16-day studies were within 10% of the target concentrations. Of the dose formulations analyzed during the 14-week studies, 20 of 22 were within 10% of the target concentrations. The out-of-range formulations were remixed and found to be within acceptable limits. Of the dose formulations analyzed during the 2-year studies, 148 of 156 for rats and 63 of 64 for mice were within 10% of the target concentration with no value greater than 113% (rats) or 114% (mice) of the target concentration. One out-of-range dose formulation for rats and one for mice were remixed. The remaining out-of-range rat dose formulations were used; this did not affect the study results. Results of periodic referee analyses performed by the analytical chemistry laboratory during the 14-week studies agreed with the results obtained by the study laboratory (Table J6).

16-Day Studies

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 11 (rats) or 13 (mice) days and were 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice were fed diets containing 0, 600, 2,000, 5,500, 17,000, or 50,000 ppm emodin for 15 (males) or 16 (females) days. Feed and water were available *ad libitum*. Rats were housed five per cage; mice were housed individually. Clinical findings were recorded twice daily, and feed consumption was recorded weekly by cage. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all animals. The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Histopathologic examinations were performed on selected gross lesions (Table 1).

14-Week Studies

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

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 11 days and were 6 weeks old on the first day of the studies. Before initiation of the studies, 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 M).

Groups of 10 male and 10 female rats and mice were fed diets containing 0, 312.5, 625, 1,250, 2,500, or 5,000 ppm emodin for 14 weeks. Additional groups of 10 male and 10 female rats designated for special studies were fed the same diets as the core study rats. Feed and water were available *ad libitum*. Rats were housed five per cage; mice were housed individually.

Clinical findings and feed consumption were recorded weekly. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Hematology and clinical chemistry analyses were performed on all special study male rats on days 5 and 22, all special study female rats on days 8 and 24, and all core study rats surviving to study termination. At all time points, animals were anesthetized with CO_2, and blood was collected from the retroorbital sinus. Erythrocyte, platelet, and leukocyte counts, hematocrit values, and hemoglobin concentrations were determined using a Technicon H•1 analyzer (Technicon Corp., Tarrytown, NY). Leukocyte differentials, reticulocyte counts, and erythrocyte, leukocyte, and platelet morphologies were determined using light microscopy. Mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentrations were calculated from the analyses for hemoglobin concentrations, hematocrit values, and erythrocyte counts. Clinical chemistry parameters were determined using the Roche Cobas Fara chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). The parameters measured are listed in Table 1.

At the end of the 14-week studies, samples were collected for sperm motility and vaginal cytology evaluations from 0, 312.5, 1,250, and 5,000 ppm rats and mice. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1987). For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all 0 and 5,000 ppm animals and animals that died early. Table 1 lists the tissues and organs routinely examined.

Paraffin sections of the kidneys (3 μm thick) from three male rats in the 0, 1,250, and 2,500 ppm groups were stained for proliferating cell nuclear antigen (PCNA) as described by Nyska *et al.* (1997). Tissue sections were incubated with a monoclonal antibody to PCNA (PC 19 antibody; Dako Corp., Carpinteria, CA) with subsequent use of avidin-biotin peroxidase (Vectastain ABC peroxidase kit; Vector Laboratories, Inc., Burlingame, CA) for the detection of antigen-antibody complex. Positive immunostaining for PCNA was identified using the chromogen 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Corp., St. Louis, MO). Tissues with a high rate of cell proliferation (e.g., duodenum, gastric mucosa, or spleen) were included in each section as a positive PCNA control. Negative controls were prepared with normal rat serum. The degree of renal cell proliferation was quantified by counting the number of tubular epithelial cells in the cortex with stained nuclei. At least 1,500 cells were scored per animal, and the labeling index (LI) was expressed as a percentage. All of the PCNA positive cells appeared to be in S phase as determined by the

degree of nuclear staining; therefore, only the LI was quantified.

2-YEAR STUDIES

Study Design

Groups of 65 male and 65 female rats were fed diets containing 0, 280, 830, or 2,500 ppm emodin for 105 weeks. Ten male and ten female rats from each exposed group were necropsied at 6 months. Blood samples were taken from five male and five female rats in each exposed group at 3, 6, and 12 months for plasma emodin concentrations; these rats and five male and five female control rats were necropsied at 12 months. Groups of 60 male mice were fed diets containing 0, 160, 312, or 625 ppm emodin for 105 weeks. Groups of 60 female mice were fed diets containing 0, 312, 625, or 1,250 ppm emodin for 105 weeks. Ten male and ten female mice from each group were necropsied at 12 months. The remaining rats and mice from each group were necropsied at the ends of the studies.

Source and Specification of Animals

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 2-year studies. Rats and mice were quarantined for 12 and 14 days, respectively, before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. At the beginning of the studies, rats were approximately 6 weeks old and mice were approximately 7 weeks old. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix M).

Animal Maintenance

Male rats were housed three per cage and female rats were housed five per cage. Male mice were housed individually and female mice were housed five per cage. Feed was available *ad libitum* except during blood collection periods. Water was available *ad libitum*. Feed consumption by cage was measured during weeks 2 and 5 for rats, on weeks 1, 2, and 4 for mice, and then monthly thereafter for rats and mice. Cages were changed twice weekly (rats and female mice) or weekly (male mice). Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix L.

Clinical Examinations and Pathology

All animals were observed twice daily. Animal weights were recorded initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies. Clinical findings were recorded monthly.

Blood was taken from the retroorbital sinus of five male and five female rats in each exposed group at 3, 6, and 12 months for plasma emodin analyses. Four different time points were selected to provide plasma concentrations soon after the period of active feeding (0800), after a period during which little or no feeding occurs (1700), at the start of active feeding (2000), and during the period of active feeding (0300). Plasma was collected from two or three males and females each at each time point. Blood samples were taken from each animal at two times at least 6 hours apart and placed in tubes containing heparin. Samples were placed on ice, centrifuged, and frozen until analysis.

Ten male and ten female rats per exposure group were designated for interim evaluation at 6 months, and five male and five female rats (including those used for plasma emodin analyses) were designated for evaluation at 12 months. Ten male and ten female mice per exposure group were designated for interim evaluation at 12 months.

Complete necropsies and microscopic examinations were performed on all rats and mice. At the 12-month interim evaluations, the left and right kidneys and the liver of rats and mice were weighed and examined microscopically. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 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 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were

entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the bone marrow, kidney, liver, and spleen of male and female rats, the pancreatic islets and prostate gland of male rats, the uterus of female rats, the kidney, liver, lung, spleen, thymus, and thyroid gland of male mice, and the kidney, pituitary gland, and thyroid gland of female mice. Step sections were made from the residual kidney wet tissue of male mice because of an apparent trend for proliferative lesions observed in the standard sections. An average of four additional sections per kidney were taken at 0.5 mm intervals.

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

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

16-Day Studies	14-Week Studies	2-Year Studies
Study Laboratory		
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
Strain and Species		
Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source		
Simonsen Laboratories, Inc. (Gilroy, CA)	Taconic Farms (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies		
Rats: 11 days Mice: 13 days	11 days	Rats: 12 days Mice: 14 days
Average Age When Studies Began		
6 weeks	6 weeks	Rats: 6 weeks Mice: 7 weeks
Date of First Exposure		
Rats: 18 April 1988 Mice: 20 April 1988	Rats: 30 October (males) or 2 November (females) 1989 Mice: 6 November 1989	Rats: 30 August 1994 Mice: 8 September 1994
Duration of Exposure		
15 days (males) or 16 days (females)	14 weeks	105 weeks
Date of Last Exposure		
Rats: 2 (males) or 3 (females) May 1988 Mice: 4 (males) or 5 (females) May 1988	Rats: 29-30 January (males) or 1-2 February (females) 1990 Mice: 5-6 February 1990	Rats: 27-30 August 1996 Mice: 5-10 September 1996
Necropsy Dates		
Rats: 2 (males) or 3 (females) May 1988 Mice: 4 (males) or 5 (females) May 1988	Rats: 29-30 January (males) or 1-2 February (females) 1990 Mice: 5-6 February 1990	Rats: 6-Month interim evaluation - 2 and 4 March 1995 12-Month interim evaluation - 29 August 1995 Terminal - 27-30 August 1996 Mice: 12-Month interim evaluation - 11-12 September 1995 Terminal - 5-10 September 1996

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

16-Day Studies	14-Week Studies	2-Year Studies
Average Age at Necropsy		
9 weeks	20 weeks	Rats: 111 weeks Mice: 111 to 112 weeks
Size of Study Groups		
5 males and 5 females	10 males and 10 females (core study) 10 males and 10 females (special study rats only)	Rats: 65 males and 65 females Mice: 60 males and 60 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 16-day studies	Same as 16-day studies
Animals per Cage		
Rats: 5 Mice: 1	Rats: 5 Mice: 1	Rats: 3 (males) or 5 (females) Mice: 1 (males) or 5 (females)
Method of Animal Identification		
Toe clip	Tail tattoo	Tail tattoo
Diet		
NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 16-day studies	NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum* except during blood collection periods, changed weekly
Water		
Tap water (Birmingham municipal supply) via Edstrom automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 16-day studies	Same as 16-day studies
Cages		
Solid-bottom polycarbonate (Lab Products, Maywood, NJ), changed twice weekly (rats) or weekly (mice)	Same as 16-day studies	Solid-bottom polycarbonate (Lab Products, Maywood, NJ), changed twice weekly (rats and female mice) or weekly (male mice)
Cage Filters		
Reemay spun-bonded polyester (Andico, Birmingham, AL), changed every 2 weeks	Same as 16-day studies	Same as 16-day studies
Bedding		
Sani-chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed twice weekly (rats) or weekly (mice)	Same as 16-day studies	Same as 16-day studies
Racks		
Stainless steel (Lab Products, Inc., Maywood, NJ), changed and rotated every 2 weeks	Same as 16-day studies	Same as 16-day studies
Animal Room Environment		
Temperature: 72° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Room air changes: 15/hour	Temperature: 72° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Room air changes: minimum 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: minimum 10/hour

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

16-Day Studies	14-Week Studies	2-Year Studies
Exposure Concentrations 0, 600, 2,000, 5,500, 17,000, or 50,000 ppm in feed, available *ad libitum*	0, 312.5, 625, 1,250, 2,500, or 5,000 ppm in feed, available *ad libitum*	Rats: 0, 280, 830, or 2,500 ppm in feed, available *ad libitum* Mice: 0, 160, 312, or 625 ppm (males) or 0, 312, 625, or 1,250 ppm (females) in feed, available *ad libitum*
Type and Frequency of Observation Animals were observed and clinical findings recorded twice daily; animals were weighed initially, weekly, and at the end of the studies. Feed consumption was recorded weekly by cage.	Observed twice daily; animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly. Feed consumption was recorded weekly by cage.	Observed twice daily; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies. Clinical findings were recorded monthly. Feed consumption was recorded during weeks 2 and 5 (rats) or weeks 1, 2, and 4 (mice), then during 1 week each month.
Method of Sacrifice CO_2 asphyxiation	CO_2 asphyxiation	CO_2 asphyxiation
Necropsy Necropsy performed on all animals. Organs weighed were heart, right kidney, liver, lungs, right testis, and thymus.	Necropsy performed on all core study animals. Organs weighed were heart, right kidney, liver, lungs, right testis, and thymus.	Necropsy performed on all animals. Organs weighed were left and right kidneys and liver of rats and mice evaluated at 12 months.
Clinical Pathology None	Blood was collected from the retroorbital sinus of special study male rats on days 5 and 22 and female rats on days 8 and 24 and from all core study rats surviving to the end of the studies for hematology and clinical chemistry analyses. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, nucleated erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials; lymphocyte and atypical lymphocyte counts ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, total bile acids	None

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

16-Day Studies	14-Week Studies	2-Year Studies
Histopathology		
Microscopic examination was performed on selected gross lesions in the kidney, liver, lymph node, mesentery, thymus, and uterus of rats and the adrenal gland, forestomach, gallbladder, kidney, liver, spleen, and thymus of mice.	In addition to gross lesions and tissue masses the following tissues were examined from 0 and 5,000 ppm animals and animals that died early: adrenal gland, bone and marrow, brain, clitoral gland, esophagus, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular, 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. For all remaining core study groups, the kidney was examined.	Complete histopathology was performed on all rats and mice at 2 years. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone and marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. Additionally, the left and right kidneys and the liver of rats and mice evaluated at 6 or 12 months were examined.
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, sperm samples were collected from 0, 312.5, 1,250, and 5,000 ppm males and evaluated for sperm count and motility. The left testis, left epididymis, and left cauda epididymis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from 0, 312.5, 1,250, and 5,000 ppm females and evaluated for the relative frequency of estrous stages and for estrous cycle length.	None
Determination of Emodin in Plasma		
None	None	At 3, 6, and 12 months, blood was collected from the retroorbital sinus of two male and two female rats at two time points (0800 and 2000) and from three male and three female rats at two other time points (1700 and 0300).

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, A5, B1, B5, C1, C5, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. 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, to 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 $B6C3F_1$ 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 are represented as 1-P with the letter N added (e.g. $P=0.99$ is presented as $P=0.01N$). For neoplasms and nonneoplastic lesions detected at the interim evaluation, the Fisher exact test (Gart *et al.*, 1979), a procedure based on the overall proportion of affected animals, was used.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between 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, toxicokinetic, spermatid,

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

Historical Control Data

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

Quality Assurance Methods

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

Genetic Toxicology

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

The genetic toxicity studies of emodin are part of a larger effort by the NTP to develop a comprehensive database that would permit 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). These short-term genetic toxicity tests were originally developed to clarify mechanisms of chemical-induced DNA damage growing out of the earlier electrophilicity/mutagenicity relationship proposed by Miller and Miller (1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). Therefore, the information obtained from these tests applies only to mutagenic carcinogens.

For mutagenic carcinogens, the combination of DNA reactivity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in multiple species and genders of rodents and at multiple tissue sites (Ashby and Tennant, 1991). Data from NTP studies show that a positive response in *Salmonella* is the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) and that there is no complementarity among the *in vitro* genetic toxicity tests (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. Although other *in vitro* genetic toxicity tests correlate less well with rodent carcinogenicity compared with the *Salmonella* test, these other tests can provide useful information on the types of DNA and

chromosomal effects induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in the acute *in vivo* bone marrow chromosome aberration test or micronucleus test 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 are associated with 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

16-DAY STUDY

Three female rats died before the end of the study (Table 2). The final mean body weights and body weight gains of males and females receiving 5,500 ppm or greater were significantly less than those of the controls; female rats in the 50,000 ppm group lost weight during the study. Feed consumption by males and females receiving 17,000 or 50,000 ppm was decreased throughout the study. Dietary concentrations of 600, 2,000, 5,500, 17,000, and 50,000 ppm emodin resulted in average daily doses of approximately 50, 170, 480, 1,400, and 3,700 mg emodin/kg body weight to males and 50, 160, 460, 1,250, and 2,000 mg/kg to females. Pink- to yellow-colored fur was noted for all male and female rats exposed to 2,000 ppm or greater; reddish-brown staining of the anal area was also noted for all male and female rats exposed to 17,000 or 50,000 ppm. Pink to yellow urine was noted in all exposed groups of females. All males and most females receiving 17,000 or 50,000 ppm had diarrhea. Emaciation and inactivity were observed in all females receiving 50,000 ppm.

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

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	123 ± 4	200 ± 6	77 ± 2		15.7	16.8
600	5/5	120 ± 5	192 ± 6	72 ± 1	96	14.6	15.6
2,000	5/5	123 ± 4	201 ± 5	77 ± 2	101	15.7	16.3
5,500	5/5	120 ± 3	175 ± 5**	55 ± 4**	88	13.4	14.9
17,000	5/5	105 ± 4*	149 ± 6**	44 ± 8**	75	10.2	12.8
50,000	5/5	120 ± 4	136 ± 6**	16 ± 7**	68	8.5	10.3
Female							
0	5/5	107 ± 3	143 ± 2	37 ± 2		11.6	11.2
600	5/5	105 ± 4	137 ± 4	32 ± 3	96	11.4	10.9
2,000	5/5	107 ± 3	138 ± 3	32 ± 1	97	11.0	10.6
5,500	5/5	106 ± 3	133 ± 1*	27 ± 2*	93	10.9	10.3
17,000	5/5	107 ± 3	117 ± 4**	10 ± 3**	82	7.4	9.4
50,000	2/5[d]	108 ± 3	88 ± 6**	−26 ± 9**	62	2.6	4.4

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 15 (males) or 16 days (females)/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Day of death: 12,14,15

Absolute kidney weights of males receiving 17,000 or 50,000 ppm were significantly less and relative weights were significantly greater than those of the controls (Table G1). Relative kidney weights of females receiving 17,000 or 50,000 ppm were significantly increased. Relative testis weights of males receiving 5,500 ppm or greater were significantly increased. Absolute thymus weights of males receiving 5,500 ppm or greater and females receiving 17,000 or 50,000 ppm and relative thymus weights of males receiving 17,000 ppm and males and females receiving 50,000 ppm were significantly decreased. Other organ weight differences were related to decreased body weights.

When examined grossly, kidneys from some of the rats in the 17,000 or 50,000 ppm groups were mottled or contained pale or yellowish foci. One male and two females exposed to 17,000 ppm and two males and five females exposed to 50,000 ppm were examined microscopically. In all of these rats, evaluation revealed suppurative inflammation, fibrosis, renal tubule epithelial hyperplasia, and renal tubule dilatation and necrosis, and the severities of these lesions generally increased with increasing exposure concentration. Often, dilated tubules contained elongated clefts in a thin proteinaceous matrix, presumably resulting from the presence of crystals of emodin or a metabolite; these crystals were removed during histologic processing.

Exposure Concentration Selection Rationale: Based on the significant reduction in body weights, weight gain, and feed consumption and the presence of histologic lesions in the kidney of male and female rats exposed to 17,000 or 50,000 ppm, these concentrations were considered too high for the 14-week study. Although the final mean body weight of male rats exposed to 5,500 ppm was 12% lower than that of the controls, the final mean body weight of females exposed to 5,500 ppm was within 7% of the control value. Moreover, no compound-related histologic lesions were observed in males and females exposed to 5,500 ppm. Therefore, 5,500 ppm was considered appropriate for the highest exposure concentration in the 14-week study in rats.

14-Week Study

All male rats survived to the end of the study; two female rats died prior to terminal sacrifice after being bled for clinical pathology analyses (Table 3). Final mean body weights and body weight gains of males exposed to 2,500 ppm or greater and females exposed to 1,250 ppm or greater were significantly less than those of the controls. During the first week of the study, feed consumption by males exposed to 2,500 or 5,000 ppm and females exposed to 5,000 ppm was less than that by the controls. Feed consumption by these groups was similar to that by the controls for the remainder of the study. Dietary concentrations of 312.5, 625, 1,250, 2,500, and 5,000 ppm emodin resulted in average daily doses of approximately 20, 40, 80, 170, and 340 mg/kg to males and females. All exposed groups of rats had an exposure concentration related body color change pattern from yellow to red, which appeared earlier in the study as the exposure concentration increased. Colored feces were observed for one male exposed to 5,000 ppm. Diarrhea and discolored bedding were observed at various times in all groups of exposed rats.

Table 3
Survival, Body Weights, and Feed Consumption of Rats in the 14-Week Feed Study of Emodin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 14
Male							
0	10/10	131 ± 4	360 ± 6	229 ± 7		14	17
312.5	10/10	126 ± 4	358 ± 4	232 ± 3	99	14	18
625	10/10	127 ± 3	360 ± 9	233 ± 7	100	14	18
1,250	10/10	128 ± 3	357 ± 6	230 ± 7	99	12	18
2,500	10/10	121 ± 3	326 ± 6**	205 ± 5**	91	10	18
5,000	10/10	130 ± 5	326 ± 6**	196 ± 4**	91	9	18
Female							
0	10/10	115 ± 2	194 ± 2	79 ± 2		11	9
312.5	10/10	112 ± 3	196 ± 3	84 ± 2	101	11	10
625	10/10	114 ± 2	194 ± 3	80 ± 2	100	11	11
1,250	10/10	113 ± 2	185 ± 2*	72 ± 3*	95	10	9
2,500	10/10	108 ± 2	176 ± 3**	68 ± 3**	91	9	10
5,000	10/10	115 ± 3	181 ± 2**	66 ± 2**	93	8	10

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

At the first time point (day 5, males; day 8, females), there was evidence of a minimal treatment-related erythrocytosis, demonstrated by increased hematocrit values, hemoglobin concentrations, and erythrocyte counts in males exposed to 1,250 ppm or greater and in females exposed to 5,000 ppm (Table F1). The erythrocytosis was transient and was no longer apparent by the second time point (day 22, males; day 24, females). In male rats, the erythrocytosis was accompanied by decreased reticulocyte counts. At week 14, increased platelet counts occurred in males and females receiving 2,500 or 5,000 ppm. Also, at week 14, increased leukocyte counts occurred in females receiving 2,500 or 5,000 ppm. The leukocytosis was characterized by increased segmented neutrophil counts. Evidence suggesting a treatment-related erythropoietic effect was demonstrated by very minimal decreases in mean cell hemoglobin values and mean cell volumes. Alterations of mean cell hemoglobin values occurred on day 24 in females receiving 5,000 ppm and at week 14 in males receiving 2,500 or 5,000 ppm and females receiving 1,250 ppm or greater. Decreased mean cell volumes on day 5 and at week 14 occurred in males receiving 5,000 ppm and in females on day 24 receiving 2,500 or 5,000 ppm. While the changes in mean cell hemoglobin and mean cell volume were suggestive of a response to treatment, the decreases were so minimal that they were not considered biologically significant.

The transient decreases in serum activity of alkaline phosphatase that occurred during the first week of the study for various male and all female exposed groups (Table F1) would be consistent with the decreased feed consumption (Table 3). Also, during the first week, there were minimal increases in alanine aminotransferase activity in males receiving 1,250 ppm or greater and females receiving 5,000 ppm. This alanine aminotransferase effect also was transient and no longer apparent on day 22 or 24. At week 14, total protein and albumin concentrations were decreased in the 2,500 and 5,000 ppm female groups. Other clinical chemistry changes were minimal and sporadic or did not demonstrate a relationship to treatment and were not considered toxicologically relevant.

Relative kidney weights were significantly increased in male rats receiving 1,250 ppm or greater, relative lung weights were significantly increased in males receiving 625 ppm or greater, and relative testis weights were increased in males receiving 2,500 or 5,000 ppm (Table G2). Increases in absolute organ weights in males were not treatment related. Relative kidney weights were significantly increased in females receiving 625 ppm or greater, and relative liver and lung weights were increased in females receiving 625 ppm or greater. The absolute kidney weight of females receiving 5,000 ppm was significantly increased, and absolute thymus weights of females receiving 2,500 or 5,000 ppm were significantly decreased. Differences in other organ weights were considered incidental and not related to emodin exposure.

No significant differences in sperm motility between the exposed and control groups were observed (Table H1). The estrous cycle length was significantly increased in females exposed to 1,250 or 5,000 ppm (Table H2).

Histologic lesions associated with emodin exposure were observed only in the kidney of males and females (Table 4). The severity of these lesions was graded on the percentage of renal tubules with hyaline droplets or abnormal amounts of pigment. Minimal severity equated to 10% or less of renal tubules affected, mild severity to 11% to 30%, moderate severity to 31% to 75%, and marked severity to 76% or greater. Coarse, intensely stained, red-orange oval pigment granules were present in the cytoplasm of cortical epithelial cells in one male exposed to 625 ppm, all males exposed to 1,250 ppm or greater, and in all groups of exposed females. The granules did not condense in tubule lumens but were present only in the cytoplasm of epithelial cells of the proximal and distal tubules of the cortex. Hyaline droplets were present in the cytoplasm of cortical epithelial cells of all exposed groups of males and in females exposed to 312.5, 625, or 1,250 ppm. In males, the severities of hyaline droplets increased to a maximum at 1,250 ppm and then decreased at higher exposure concentrations. In females, the severities increased with increasing exposure concentration in the affected groups. No significant differences were noted in the mean labeling index among any of the proliferating cell nuclear antigen-stained groups.

TABLE 4
Incidences of Nonneoplastic Lesions and Mean Labeling Indices of the Kidney in Rats in the 14-Week Feed Study of Emodin

	0 ppm	312.5 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Male						
Number Examined Microscopically	10	10	10	10	10	10
Renal Tubule Hyaline Droplet[a]	0	10** (2.3)[b]	10** (2.4)	10** (3.0)	10** (1.4)	10** (1.8)
Renal Tubule Pigmentation	0	0	1 (1.0)	10** (1.0)	10** (1.5)	10** (2.0)
Proliferating Cell Nuclear Antigen Staining[c]	3	0	0	3	0	3
Mean Labeling Index (%)	0.34			0.40		0.45
Female						
Number Examined Microscopically	10	10	10	10	10	10
Renal Tubule Hyaline Droplet	0	10** (1.2)	10** (1.1)	10** (1.9)	0	0
Renal Tubule Pigmentation	0	10** (1.0)	10** (1.1)	10** (1.5)	10** (1.0)	10** (2.0)

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

Exposure Concentration Selection Rationale: The responses of male and female rats exposed to 2,500 or 5,000 ppm were very similar. Mean body weights of groups exposed to 2,500 or 5,000 ppm were less than the control (7% to 9%) and other exposed groups throughout the 14-week study. Additionally, kidney lesions were very similar between these two groups although the severity of pigmentation was greater at 5,000 ppm than at 2,500 ppm. However, the observed kidney lesions were not considered the type that would affect survival in a long-term study. Because the responses at 2,500 and 5,000 ppm were very similar and the use of 5,000 ppm in a long-term study would have increased the potential for toxicity, 2,500 ppm was considered adequate as the highest exposure concentration for the 2-year study. The lower exposure concentrations selected were 280 and 830 ppm.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 1). Survival of all exposed groups of male and female rats was similar to that of the control groups.

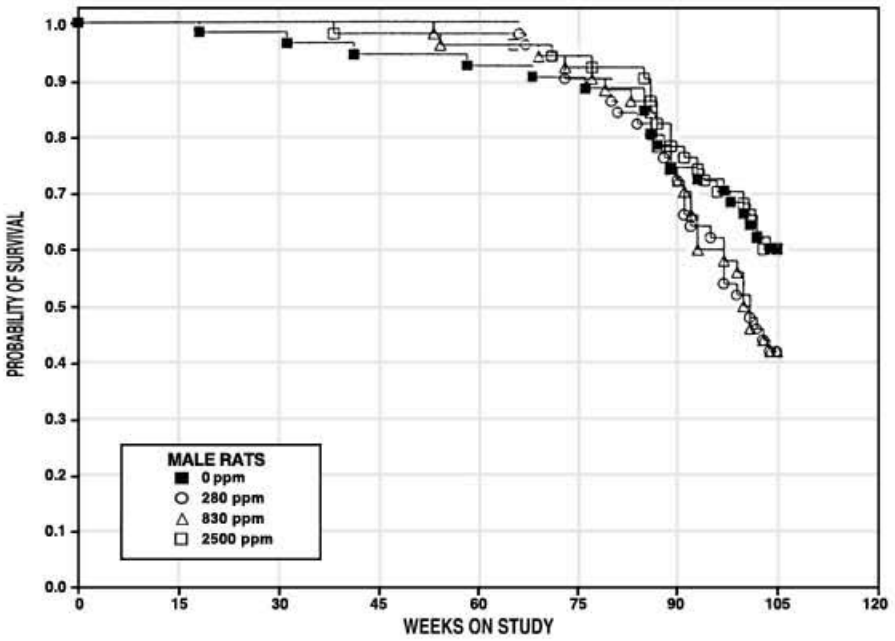
Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of rats in the 2,500 ppm groups were less than those of controls beginning at week 2 of the study (Tables 6 and 7 and Figure 2). Feed consumption by exposed groups was similar to that by the controls throughout the study (Tables K1 and K2). Dietary concentrations of 280, 830, and 2,500 ppm delivered average daily doses of approximately 110, 320, and 1,000 mg/kg to males and 120, 370, and 1,100 mg/kg to females. There were no clinical findings that could be attributed to emodin exposure.

TABLE 5
Survival of Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
Male				
Animals initially in study	65	65	65	65
6-Month interim evaluation[a]	10	10	10	10
12-Month interim evaluation[a]	5	5	5	5
Moribund	17	26	27	16
Natural deaths	3	3	2	4
Animals surviving to study termination	30	21	21	30
Percent probability of survival at end of study[b]	60	42	42	60
Mean survival (days)[c]	659	665	663	680
Survival analysis[d]	P=0.396N	P=0.145	P=0.154	P=1.000N
Female				
Animals initially in study	65	65	65	65
6-Month interim evaluation	10	10	10	10
12-Month interim evaluation	5	5	5	5
Moribund	12	8	11	15
Natural deaths	5	3	4	1
Animals surviving to study termination	33	39	35	34
Percent probability of survival at end of study	66	78	70	68
Mean survival (days)	695	704	699	693
Survival analysis	P=0.762	P=0.285N	P=0.761N	P=1.000N

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored 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**.



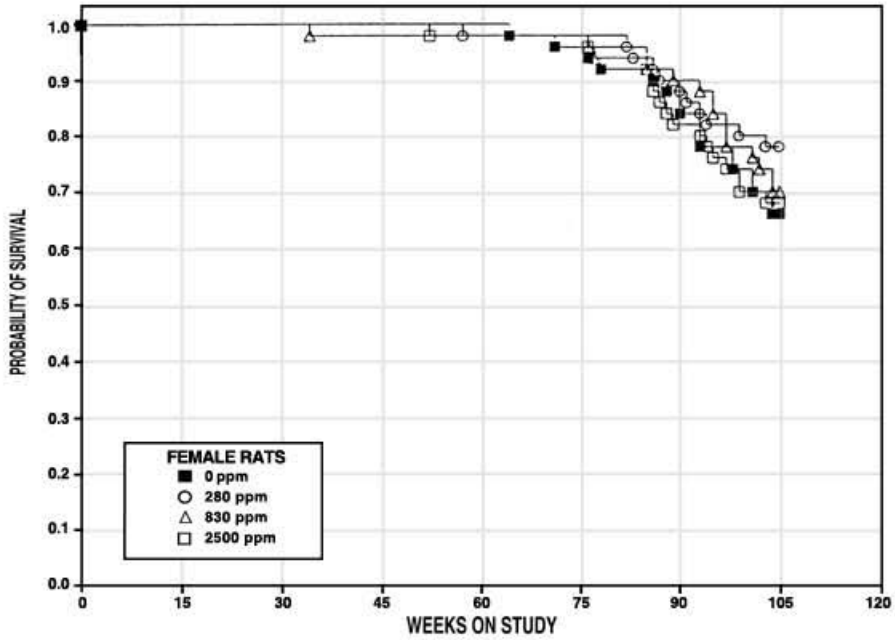


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Emodin in Feed for 2 Years

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

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	280 ppm Av. Wt. (g)	280 ppm Wt. (% of controls)	280 ppm No. of Survivors	830 ppm Av. Wt. (g)	830 ppm Wt. (% of controls)	830 ppm No. of Survivors	2,500 ppm Av. Wt. (g)	2,500 ppm Wt. (% of controls)	2,500 ppm No. of Survivors
1	139	60	135	97	60	134	97	60	133	96	60
2	178	60	175	98	60	171	96	60	160	90	60
3	214	60	208	97	60	204	95	60	192	90	60
4	242	60	235	97	60	230	95	60	211	87	60
5	264	60	260	99	60	253	96	60	236	90	60
6	283	60	278	98	60	269	95	60	253	90	60
7	299	60	294	98	60	286	96	60	269	90	60
8	313	60	307	98	60	298	95	60	278	89	60
9	325	60	319	98	60	310	96	60	291	89	60
10	328	60	323	99	60	321	98	60	302	92	60
11	342	60	338	99	60	330	96	60	314	92	60
12	352	60	345	98	60	336	96	60	319	91	60
13	359	60	356	99	60	344	96	60	330	92	60
17	386	60	382	99	60	373	97	60	356	92	60
21	405	59	400	99	60	390	96	60	369	91	60
25	420	59	416	99	60	404	96	60	387	92	60
29[a]	438	49	431	98	50	423	97	50	398	91	50
33	448	48	443	99	50	434	97	50	412	92	50
37	461	48	457	99	50	445	97	50	427	93	50
41	464	48	457	99	50	449	97	50	431	93	49
45	467	47	465	100	50	458	98	50	436	94	49
49	471	47	468	100	50	460	98	50	440	94	49
53	473	47	469	99	50	458	97	50	441	93	49
57	474	47	472	100	50	464	98	48	444	94	49
61	472	46	468	99	50	459	97	48	440	93	49
65	471	46	465	99	50	458	97	48	440	94	49
69	472	45	465	99	48	459	97	48	440	93	48
73	471	45	464	99	47	457	97	47	443	94	47
77	471	44	464	99	45	457	97	45	442	94	47
81	472	44	462	98	42	454	96	44	443	94	46
85	465	44	460	99	41	456	98	43	443	95	46
89	459	38	449	98	37	444	97	38	434	95	39
93	456	37	445	98	32	431	95	33	430	94	38
97	452	36	432	96	31	429	95	30	427	95	35
101	449	33	427	95	26	420	94	25	417	93	34
Mean for weeks											
1-13	280		275	98		268	96		253	91	
14-52	440		435	99		426	97		406	92	
53-101	466		457	98		450	97		437	94	

[a] Interim evaluation occurred during week 27.

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

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	280 ppm Av. Wt. (g)	280 ppm Wt. (% of controls)	280 ppm No. of Survivors	830 ppm Av. Wt. (g)	830 ppm Wt. (% of controls)	830 ppm No. of Survivors	2,500 ppm Av. Wt. (g)	2,500 ppm Wt. (% of controls)	2,500 ppm No. of Survivors
1	111	60	109	99	60	109	98	60	108	97	60
2	131	60	129	99	60	128	98	60	124	95	60
3	145	60	141	97	60	139	96	60	134	93	60
4	153	60	150	99	60	148	97	60	142	93	60
5	163	60	161	99	60	157	96	60	152	93	60
6	169	60	165	98	60	163	96	60	157	93	60
7	176	60	173	98	60	170	97	60	164	93	60
8	180	60	176	98	60	175	97	60	170	94	60
9	183	60	180	98	60	177	97	60	173	95	60
10	185	60	183	99	60	180	97	60	176	95	60
11	190	60	187	99	60	185	98	60	180	95	60
12	192	60	190	99	60	187	98	60	185	96	60
13	195	60	192	99	60	189	97	60	186	95	60
17	205	60	203	99	60	199	97	60	198	97	60
21	211	60	209	99	60	205	97	60	202	96	60
25	219	60	214	98	60	211	96	60	209	95	60
29[a]	226	50	222	98	50	217	96	50	217	96	50
33	235	50	228	97	50	226	96	50	222	95	50
37	241	50	233	97	50	230	96	49	224	93	50
41	247	50	240	97	50	237	96	49	232	94	50
45	255	50	247	97	50	243	95	49	237	93	50
49	265	50	253	96	50	250	95	49	245	93	50
53	268	50	256	95	50	253	94	49	247	92	49
57	274	50	263	96	50	260	95	49	253	93	49
61	282	50	269	95	49	268	95	49	260	92	49
65	292	49	277	95	49	278	95	49	272	93	49
69	301	49	286	95	49	287	95	49	279	93	49
73	308	48	293	95	49	294	95	49	289	94	49
77	319	47	302	95	49	300	94	48	296	93	48
81	324	46	303	93	49	308	95	47	296	91	48
85	331	46	316	96	47	314	95	47	304	92	47
89	333	44	317	95	45	318	96	45	302	91	42
93	330	40	319	97	43	317	96	45	308	93	41
97	336	39	326	97	41	320	95	42	310	92	38
101	336	37	331	98	40	331	98	39	314	94	35
Mean for weeks											
1-13	167		164	99		162	97		158	94	
14-52	234		228	98		224	96		221	95	
53-101	310		297	96		296	95		287	93	

[a] Interim evaluation occurred during week 27.

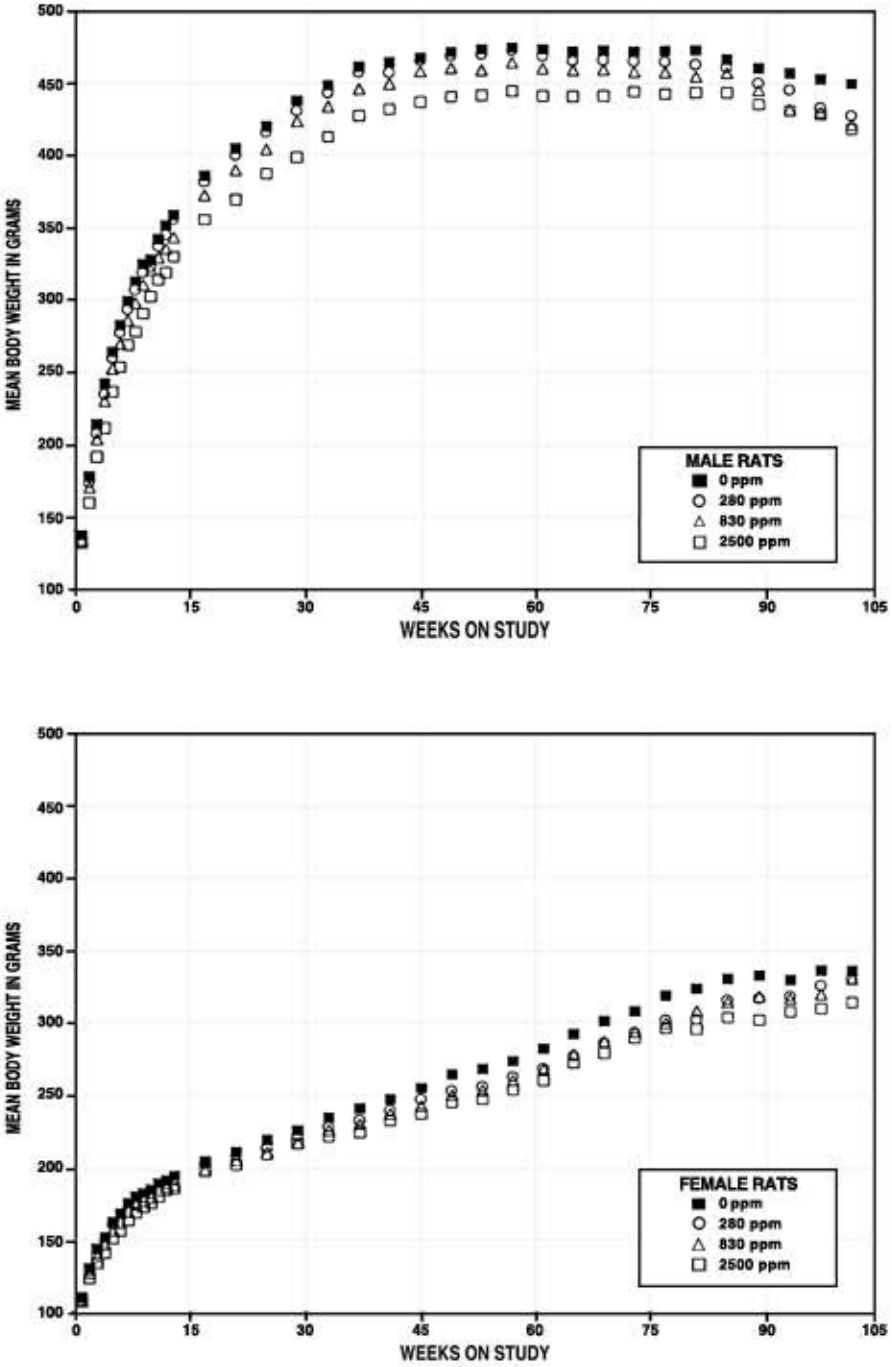


FIGURE 2
Growth Curves for Male and Female Rats Exposed to Emodin in Feed for 2 Years

Determinations of Emodin in Plasma

The mean concentrations of emodin in plasma determined at 3, 6, and 12 months in exposed rats are presented in Appendix I. Four different time points were selected to provide plasma concentrations soon after the period of active feeding (0800), after a period during which little or no feeding occurs (1700), at the start of active feeding (2000), and during the period of active feeding (0300). Plasma was collected from two or three males and females at each time point. Plasma levels at 6 months were generally lower than those at 3 months in the 2,500 ppm group; levels at 12 months were the lowest. Plasma levels between 0800 and 1700 were reduced by one-third to one-half in most groups. This reduction occurred more slowly than might be expected with a 2- to 4-hour terminal phase half-life; this may be due in part to a lack of homogeneity in the times when the animals ceased their nighttime feeding.

Pathology and Statistical Analyses

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

Zymbal's Gland: Zymbal's gland carcinomas were noted in three female rats exposed to 2,500 ppm (0 ppm, 0/50; 280 ppm, 0/50; 830 ppm, 0/50; 2,500 ppm, 3/50; Table B3). Two of the carcinomas were well differentiated, containing neoplastic sebaceous cells and squamous epithelium with keratin; the third carcinoma consisted of poorly differentiated, highly invasive neoplastic islands. This range of morphology is typically described in spontaneous neoplasms in rats (Seely, 1991). Single cases of the same neoplasm were also seen in one male rat exposed to 280 ppm and in one 2,500 ppm male rat (0/50, 1/50, 0/50, 1/50; Table A1). The incidence of carcinoma in 2,500 ppm females exceeded the range in historical controls in 2-year NTP feed studies (Table B4a). The historical incidence for Zymbal's gland carcinoma in untreated male rats is 9/904 (1.0% ± 1.2%; range, 0%-4%).

Pituitary Gland: The incidences of adenoma of the pars distalis in female rats were significantly increased in the 830 and 2,500 ppm groups compared to the control group (15/49, 21/50, 25/49, 25/49; Table B3). However, the incidences in the exposed groups were similar to the historical control incidence of 443/893 (49.5% ± 12.0%; range, 33%-72%). The significantly increased incidences appear to be related to the unusually low incidence in the concurrent control group (31%), which is just below the range in historical controls, rather than an effect of exposure to emodin. Additionally, there was no increase in the incidence of hyperplasia (13/49, 10/50, 3/49, 5/49; Table B5) in females and no evidence of an increase in the incidence of proliferative lesions in male rats (Tables A1 and A5). For these reasons, the increased incidences of pars distalis adenomas in females were not considered to be exposure related.

Nose: Squamous cell carcinomas were noted in the nasal cavity of two male rats exposed to 2,500 ppm (0/50, 0/50, 0/50, 2/50; Table A1). The carcinomas were located at the first and second levels of the nasal cavity. In one rat, the carcinomas appeared to originate from the nasal septa, while in the other animal, the carcinoma seemed to originate from the maxilloturbinate and the base of the nasal cavity. In both cases, the carcinomas were well differentiated, showing keratinization and formation of typical keratin pearls. Extensive invasion into the surrounding underlying tissue was evident, as was superficial secondary inflammation. Squamous cell carcinomas are rare in F344/N rats; the historical control range for squamous cell carcinomas in feed studies is 0% to 2% (Table A4a). However, no other lesions were present in the nose. The absence of preneoplastic lesions or other chemical-related lesions is consistent with an absence of response to chemical exposure in the nose. Therefore, the two squamous cell carcinomas were considered unrelated to emodin exposure.

Kidney: At the 6- and 12-month interim evaluations and at 2 years, emodin-related increases in the incidences of hyaline droplets in renal tubule epithelial cells in the kidney were observed in all exposed groups (Tables 8, A5, and B5). The incidences of renal tubule pigmentation were significantly increased in all exposed groups of males at 2 years. The severities of these lesions ranged from minimal to moderate and generally increased with increasing exposure concentration. (Severity was graded using the criteria discussed for the 14-week study.)

At the end of the 2-year study, renal tubule hyaline droplets were observed in males and females in all exposed groups; a dose-related, increased severity was more evident among the female groups. The hyaline droplets consisted of variably sized, spherical, homogenous eosinophilic to light brown pigmented globules, 3 to 15 μ in diameter. The globules were located within the cytoplasm of the proximal tubule epithelium (Plate 1). In the controls, the globules were limited to the deeper cortex, while in the exposed groups the globules extended to the majority of the cortex (Plate 2). The globules were similar to those noted in the 14-week study. At the interim evaluations, the hyaline droplets in males generally were eosinophilic with smaller numbers of brown droplets; in females, the hyaline droplets commonly

TABLE 8
Incidences of Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
Male				
6-Month Interim Evaluation				
Number Examined Microscopically	10	10	10	10
Renal Tubule Hyaline Droplet[a]	0	10** (2.4)[b]	10** (2.8)	10** (2.5)
Renal Tubule Pigmentation	10 (1.0)	10 (1.0)	10 (1.0)	10 (1.9)
12-Month Interim Evaluation				
Number Examined Microscopically	5	5	5	5
Renal Tubule Hyaline Droplet	0	5** (2.2)	5** (2.6)	5** (2.8)
Renal Tubule Pigmentation	5 (1.0)	5 (1.0)	5 (1.0)	4 (1.3)
2-Year Study				
Number Examined Microscopically	50	50	50	50
Renal Tubule Hyaline Droplet	3 (1.0)	45** (2.0)	43** (1.8)	43** (1.7)
Renal Tubule Pigmentation	35 (1.3)	47** (1.8)	49** (1.8)	50** (2.1)
Female				
6-Month Interim Evaluation				
Number Examined Microscopically	10	10	10	10
Renal Tubule Hyaline Droplet	6 (1.0)	10* (1.0)	10* (1.3)	10* (2.4)
Renal Tubule Pigmentation	10 (1.0)	10 (1.0)	10 (1.3)	10 (2.5)
12-Month Interim Evaluation				
Number Examined Microscopically	5	5	5	5
Renal Tubule Hyaline Droplet	3 (1.7)	5 (2.6)	5 (2.4)	5 (3.0)
Renal Tubule Pigmentation	5 (1.4)	5 (2.0)	5 (1.8)	5 (3.0)
2-Year Study				
Number Examined Microscopically	49	50	49	50
Renal Tubule Hyaline Droplet	22 (1.6)	49** (2.1)	49** (2.6)	50** (3.0)
Renal Tubule Pigmentation	45 (1.2)	49 (1.4)	49 (2.4)	50 (3.0)

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

were brown with smaller numbers of eosinophilic droplets. At 2 years, the droplets were commonly brown. The globules were positive for PAS but negative for iron (Gomori's Prussian blue), bile pigment (Hall's stain), lipofuscin (Schmorl's stain), and protein (Mallory-Heidenhain) (Plate 3). Most of the changes identified in the kidney of rats were not consistent with those generally observed with α2u nephropathy. There was no obvious exacerbation of nephropathy as is generally associated with α2u induction. Linear mineralization of the renal medulla, sometimes associated with α2u nephropathy, was not observed in this study.

Exposure concentration-related increases in the severities of renal tubule pigmentation were noted in all exposed groups of males and females. The pigmentation had the same morphologic characteristics as described in the 14-week study in rats. The pigment consisted of fine to coarse brown granules in the cytoplasm of renal tubule epithelial cells and interstitial macrophages of affected tubules. These pigmented

deposits were primarily located near the level of the corticomedullary junction. The pigmented granules stained negative for PAS and iron (Prussian blue).

Mononuclear Cell Leukemia: There were negative trends in the incidences of mononuclear cell leukemia in male and female rats (Tables 9, A3, and B3), and the incidences in the 2,500 ppm groups were significantly less than those in the controls. The decreased incidences were particularly evident in the liver, spleen, and bone marrow, which are among the organs most frequently affected by mononuclear cell leukemia. The incidence in females exposed to 2,500 ppm was below the historical control range (Table B4b); the incidence in 2,500 ppm males was at the lower end of the historical control range (Table A4b).

Other Organs: There were increased incidences of clear cell and eosinophilic foci of the liver and hematopoietic cell proliferation and pigmentation (hemosiderin) of the spleen in females exposed to 2,500 ppm (Tables 10 and B5). The incidence of bone marrow hyperplasia in males exposed to 2,500 ppm was increased (Tables 10 and A5). Liver foci and splenic hematopoietic cell proliferation and pigmentation are common spontaneous changes observed in rats. Often, in severe cases of mononuclear cell leukemia, these spontaneous changes either do not occur or are not recognized histologically. This is because severe mononuclear cell leukemia causes significant alterations in the architecture of the livers and spleens that mask the spontaneous lesions. In the present study, the incidences of mononuclear cell leukemia were significantly decreased in males and females exposed

TABLE 9
Incidences of Mononuclear Cell Leukemia in Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
Male				
Mononuclear Cell Leukemia[a]				
Overall rate[b]	28/50 (56%)	31/50 (62%)	29/50 (58%)	18/50 (36%)
Adjusted rate[c]	61.5%	65.1%	63.7%	38.7%
Terminal rate[d]	16/30 (53%)	7/21 (33%)	11/21 (52%)	6/30 (20%)
First incidence (days)	284	491	373	266
Poly-3 test[e]	P=0.003N	P=0.442	P=0.500	P=0.021N
Female				
Mononuclear Cell Leukemia[f]				
Overall rate	14/50 (28%)	17/50 (34%)	16/50 (32%)	3/50 (6%)
Adjusted rate	30.1%	35.6%	33.5%	6.7%
Terminal rate	7/33 (21%)	12/39 (31%)	9/35 (26%)	1/34 (3%)
First incidence (days)	448	575	527	603
Poly-3 test	P<0.001N	P=0.361	P=0.445	P=0.003N

[a] Historical incidence for 2-year feed studies with untreated control groups (mean ± standard deviation): 494/904 (54.7% ± 11.2%); range, 32%-74%
[b] Number of animals with neoplasm per number of animals necropsied
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence are the P values 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**.
[f] Historical incidence: 261/901 (29.0% ± 7.8%); range, 16%-42%

TABLE 10
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Feed Study of Emodin

	0 ppm		280 ppm		830 ppm		2,500 ppm	
Male								
Liver[a]	50		50		50		50	
Clear Cell Focus[b]	7		7		9		13	
Eosinophilic Focus	6		8		6		10	
Bile Duct, Hyperplasia	33	(2.4)[c]	24	(2.7)	20**	(2.6)	14**	(2.2)
Spleen	50		50		50		50	
Hematopoietic Cell Proliferation	3	(3.0)	5	(2.2)	4	(2.8)	8	(2.3)
Pigmentation	1	(3.0)	2	(3.0)	4	(3.0)	4	(3.3)
Bone Marrow	50		50		50		50	
Hyperplasia	3	(2.7)	5	(2.6)	3	(3.0)	11*	(2.8)
Female								
Liver	49		50		50		50	
Clear Cell Focus	2		10*		4		11**	
Eosinophilic Focus	18		10		18		27*	
Spleen	50		50		50		50	
Hematopoietic Cell Proliferation	27	(1.7)	24	(1.6)	31	(1.6)	40**	(1.7)
Pigmentation	30	(2.6)	30	(2.9)	35	(2.7)	43**	(2.8)

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

to 2,500 ppm, making it easier to observe the spontaneous changes in the liver and spleen. Therefore, the increased incidences of liver and spleen lesions observed in females exposed to 2,500 ppm are considered secondary to the low incidence of mononuclear cell leukemia in this group. Because the bone marrow is also commonly involved in mononuclear cell leukemia, the marginal increase in the incidence of hyperplasia in males exposed to 2,500 ppm could also be explained by the decreased incidence of mononuclear cell leukemia in this group.

The incidences of bile duct hyperplasia decreased with exposure concentration in male rats. Bile duct hyperplasia is a common spontaneous change in rats but also can be secondary to liver damage caused by the presence of mononuclear cell leukemia. The decreased incidence of bile duct hyperplasia in 2,500 ppm males could be explained by the reduced incidence of mononuclear cell leukemia in this group; the decreased incidences of bile duct hyperplasia in the 280 and 830 ppm groups suggest that the mononuclear cell leukemia was not as advanced.

MICE

16-DAY STUDY

All mice administered 50,000 ppm died before the end of the study, and mice in the 17,000 ppm groups lost weight during the study (Table 11). Feed consumption by 5,550 ppm females was greater than that by the controls throughout the study. Dietary concentrations of 600, 2,000, 5,500, and 17,000 ppm emodin resulted in average daily doses of approximately 120, 400, 1,200, and 3,800 mg/kg to males and 140, 530, 1,600, and 5,000 mg/kg to females. The average daily doses for males and females exposed to 50,000 ppm were not calculated due to high mortality. Yellow or orange urine was observed in all exposed groups. Orange or yellow and orange fur was noted in groups receiving 5,500 ppm or greater. Females in the 5,500 and 17,000 ppm groups were emaciated. Diarrhea was noted for two males receiving 17,000 ppm and for one female each in the 5,500, 17,000, and 50,000 ppm groups.

TABLE 11
Survival, Mean Body Weights, and Feed Consumption of Mice in the 16-Day Feed Study of Emodin

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	24.0 ± 0.4	26.5 ± 0.5	2.5 ± 0.3		5.4	5.3
600	5/5	23.9 ± 0.5	26.0 ± 0.8	2.1 ± 0.4	98	5.0	5.1
2,000	5/5	23.6 ± 0.4	26.0 ± 0.9	2.4 ± 0.6	98	4.8	5.1
5,500	5/5	23.2 ± 0.5	24.6 ± 0.5	1.4 ± 0.6	93	5.4	5.1
17,000	5/5	23.0 ± 1.0	21.7 ± 0.5**	−1.3 ± 0.7**	82	4.4	5.4
50,000	0/5[d]	21.4 ± 1.1	—	—	—	—	—
Female							
0	5/5	18.4 ± 0.2	21.5 ± 0.2	3.0 ± 0.2		4.9	5.3
600	5/5	18.5 ± 0.5	21.4 ± 0.2	2.9 ± 0.4	100	4.3	5.5
2,000	5/5	18.2 ± 0.4	21.5 ± 0.3	3.3 ± 0.4	100	5.3	5.8
5,500	5/5	18.6 ± 0.5	20.5 ± 0.7	1.9 ± 0.3	95	5.2	6.2
17,000	5/5	18.2 ± 0.2	16.7 ± 0.8**	−1.5 ± 0.9**	78	4.7	5.5
50,000	0/5[e]	18.3 ± 0.1	—	—	—	—	—

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number of animals surviving at 15 (males) or 16 days (females)/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No final mean body weights were calculated for groups with 100% mortality.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Day of death: 2,2,5,5,6
[e] Day of death: 4,4,4,5,5

The relative testis weight of 17,000 ppm males and the relative kidney weight of 17,000 ppm females were significantly greater than those of the controls; absolute and relative thymus weights of 5,500 and 17,000 ppm males and females were significantly less (Table G4). Other organ weight differences were related to decreased body weights.

The gallbladders of mice from the 17,000 ppm group contained pale particulate material that was grossly visible. Microscopic examination of the gallbladders revealed the presence of golden-yellow elongate (needle-like) crystals. Inflammation was present in gallbladders from the 5,500, 17,000, and 50,000 ppm groups. Crystalline material within the renal tubules and an associated inflammation, necrosis, and fibrosis were present at 5,500, 17,000, and 50,000 ppm.

Exposure Concentration Selection Rationale: Based on 100% mortality at 50,000 ppm and reduced body weights and kidney lesions that occurred in the 17,000 ppm groups, these exposure concentrations were considered too high for the 14-week study; 5,000 ppm was selected as the highest exposure concentration.

14-Week Study

All mice survived to the end of the study (Table 12). Final mean body weights and body weight gains of males exposed to 2,500 or 5,000 ppm were significantly less than those of the controls. Feed consumption by exposed groups was generally similar to that by controls. Dietary concentrations of 312.5, 625, 1,250, 2,500, and 5,000 ppm emodin resulted in average daily doses of approximately 50, 100, 190, 400, and 800 mg/kg to males and 60, 130, 240, 500, and 1,100 mg/kg to females. In all exposed groups, color changes were noted in the urine, skin, and fur. Urine and urine stains were orange or more often red with increasing exposure concentration. In male mice, colored feces were noted in groups exposed to 312.5, 2,500, or 5,000 ppm, and diarrhea was recorded for groups exposed to 2,500 or 5,000 ppm. Colored feces and diarrhea were noted in groups of females receiving 1,250 ppm or greater.

Relative kidney weights of male mice exposed to 1,250 ppm or greater, relative lung weights of males exposed to 625 ppm or greater, and relative liver weights of female mice exposed to 625 ppm or greater were increased compared to the control groups (Table G5). Differences in relative weights of other organs were attributed to body weight differences.

Table 12
Survival, Body Weights, and Feed Consumption of Mice in the 14-Week Feed Study of Emodin

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 14
Male							
0	10/10	24.0 ± 0.4	37.2 ± 1.0	13.3 ± 0.8		4.8	4.3
312.5	10/10	23.9 ± 0.3	36.3 ± 0.6	12.4 ± 0.5	98	5.0	4.2
625	10/10	23.5 ± 0.4	35.4 ± 0.5	11.9 ± 0.5	95	4.5	4.5
1,250	10/10	24.2 ± 0.4	37.4 ± 0.6	13.2 ± 0.6	100	5.0	4.5
2,500	10/10	23.2 ± 0.6	33.8 ± 0.4**	10.6 ± 0.5**	91	4.8	4.3
5,000	10/10	23.4 ± 0.5	34.1 ± 0.7**	10.7 ± 0.7**	92	5.3	4.4
Female							
0	10/10	18.7 ± 0.3	29.3 ± 0.8	10.5 ± 0.7		4.8	4.4
312.5	10/10	18.5 ± 0.2	29.6 ± 0.7	11.1 ± 0.6	101	4.5	5.0
625	10/10	18.6 ± 0.3	32.0 ± 1.0	13.4 ± 0.9*	109	5.5	5.3
1,250	10/10	18.7 ± 0.3	29.6 ± 0.7	10.9 ± 0.5	101	5.1	4.8
2,500	10/10	18.6 ± 0.1	30.4 ± 0.7	11.8 ± 0.7	104	4.7	4.4
5,000	10/10	18.4 ± 0.2	27.9 ± 0.6	9.5 ± 0.5	95	5.0	5.1

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

No significant differences in sperm motility or in vaginal cytology parameters between exposed and control groups were observed (Tables H3 and H4).

Histopathologic changes related to emodin exposure were confined to the kidney and involved the renal tubules (Table 13; severity was graded using the criteria discussed for the 14-week rat study). The incidences and severities of nephropathy were increased in groups exposed to 1,250 ppm or greater. Nephropathy, resembling the nephropathy commonly observed as a minor background lesion in untreated control mice, was more severe and frequent in the emodin-treated male mice. Nephropathy consisted of a focal or segmental lesion, principally involving the cortex, characterized by tubules that were either lined by flattened hyperchromatic epithelial cells or by hyperplastic epithelium, thickened basement membranes, and proliferation of fibrous connective tissue of the interstitium. Glomeruli had thickened basement membranes and hypertrophy and hyperplasia of the epithelium lining Bowman's capsule. Lumenal, obstructive, brownish-red to almost black deposits were noted in the renal cortical and collecting tubules in all exposed groups of males and in females exposed to 1,250 ppm or greater, and the severities of renal tubule pigmentation generally increased with increasing exposure concentration.

Exposure Concentration Selection Rationale: Exposure concentration selection for the 2-year mouse study was based primarily on the response observed in the kidney. Pigmentation was present in the kidneys of all exposed groups of males, and both the incidence and severity increased with increasing exposure concentration. Minimal nephropathy was present in one control male, one male from the 312.5 ppm group, and in one male from the 625 ppm group. However, at 1,250 ppm the incidence and severity of nephropathy and the incidence of pigmentation increased sharply. Based on this response, 1,250 ppm was considered too high for a 2-year study, and the highest exposure concentration chosen for male mice was 625 ppm with lower exposure concentrations of 312 and 160 ppm.

Nephropathy and pigmentation of the kidney were present in female mice receiving 1,250 ppm or greater; however, the incidence and severity were sharply increased only in the 5,000 ppm group. Because the responses at 1,250 and 2,500 ppm were very similar and the potential for cumulative toxicity was less at 1,250 ppm, the highest exposure concentration chosen for the 2-year study in female mice was 1,250 ppm. The lower exposure concentrations selected were 625 and 312 ppm.

TABLE 13
Incidences of Nonneoplastic Lesions of the Kidney in Mice in the 14-Week Feed Study of Emodin

	0 ppm	312.5 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Male						
Number Examined Microscopically	10	10	10	10	10	10
Nephropathy[a]	1 (1.0)[b]	1 (1.0)	1 (1.0)	7** (2.1)	10** (2.1)	9** (2.3)
Renal Tubule Pigmentation	0	3 (1.0)	4* (1.3)	8** (1.3)	10** (1.8)	10** (1.8)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Nephropathy	1 (1.0)	0	0	3 (1.0)	4 (1.3)	10** (1.6)
Renal Tubule Pigmentation	0	0	0	5* (1.2)	5* (1.4)	9** (1.7)

* 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

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 3). Survival of all exposed groups of male and female mice was similar to that of the control groups.

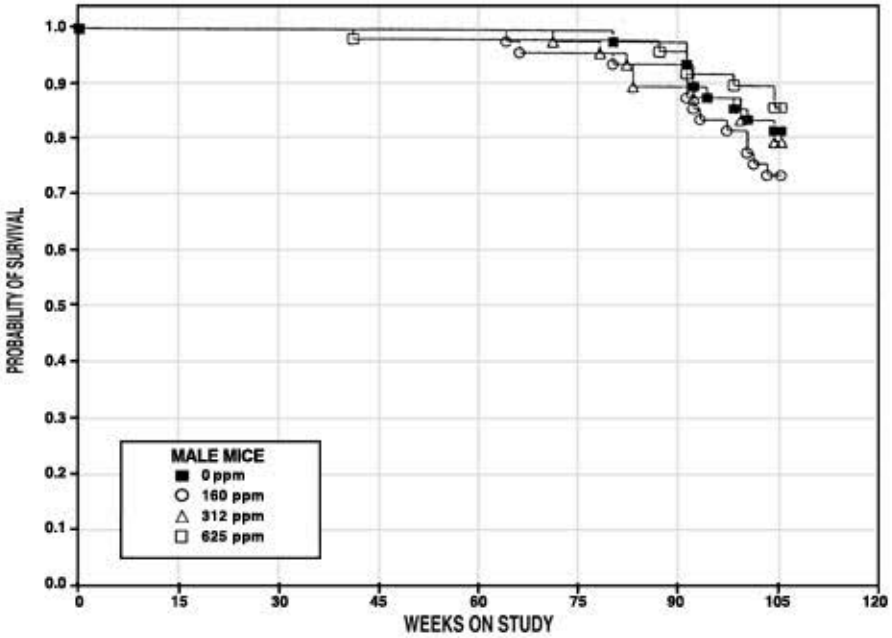
Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of exposed mice were similar to those of control mice throughout the study (Tables 15 and 16 and Figure 4). No differences in feed consumption were noted between exposed and control control groups (Tables K3 and K4). Dietary concentrations of 160, 312, and 625 ppm delivered average daily doses of approximately 15, 35, and 70 mg/kg to males. Dietary concentrations of 312, 625, and 1,250 ppm delivered average daily doses of approximately 30, 60, and 120 mg/kg to females. There were no clinical findings that could be attributed to emodin exposure.

Table 14
Survival of Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
Male				
Animals initially in study	60	60	60	60
12-Month interim evaluation[a]	10	10	10	10
Moribund	4	6	6	7
Natural deaths	5	7	4	
Animals surviving to study termination	41	37	40	43
Percent probability of survival at end of study[b]	82	74	80	86
Mean survival (days)[c]	715	702	708	713
Survival analysis[d]	P=0.471N	P=0.428	P=0.949	P=0.796N
	0 ppm	**312 ppm**	**625 ppm**	**1,250 ppm**
Female				
Animals initially in study	60	60	60	60
12-Month interim evaluation	10	10	10	10
Moribund	7	9	2	7
Natural deaths	6	2	7	7
Accidental deaths			1	
Animals surviving to study termination	37	39	40	36
Percent probability of survival at end of study	74	78	82	72
Mean survival (days)	702	701	707	704
Survival analysis	P=0.880	P=0.805N	P=0.405N	P=1.000

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored 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**.



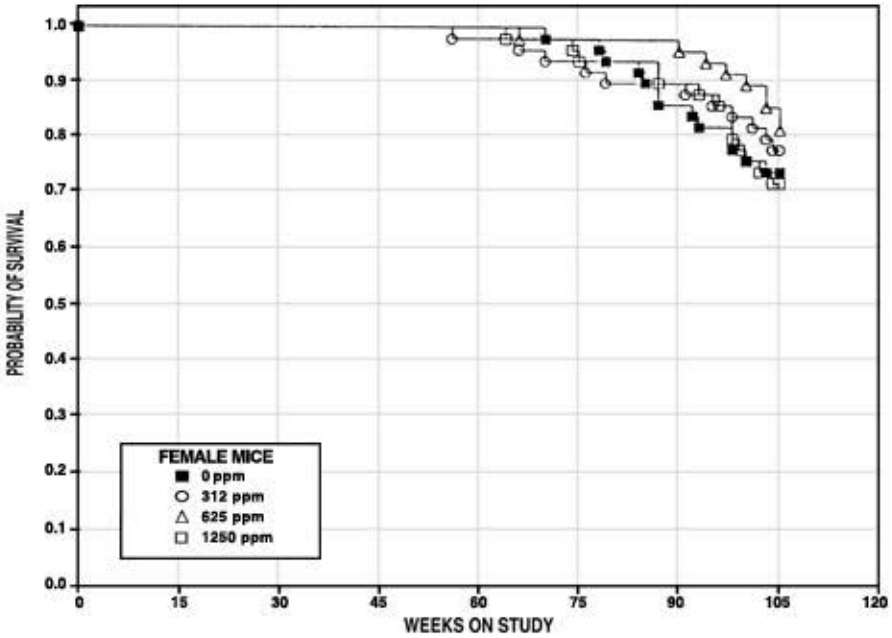


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Emodin in Feed for 2 Years

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

Weeks on Study	0 ppm		160 ppm			312 ppm			625 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	23.8	60	23.8	100	60	24.0	101	60	23.5	99	60
2	25.7	60	25.5	99	60	25.7	100	60	25.5	99	60
3	27.1	60	26.9	99	60	26.8	99	60	26.9	99	60
4	27.8	60	27.4	99	60	27.6	99	60	27.5	99	60
5	28.7	60	28.3	99	60	28.6	100	60	28.5	99	60
6	29.6	60	29.4	99	60	29.5	100	60	29.3	99	60
7	30.3	60	30.2	100	60	30.6	101	60	30.2	100	60
8	31.4	60	31.2	99	60	31.3	100	60	31.2	99	60
9	31.7	60	31.3	99	60	31.6	100	60	31.4	99	60
10	32.4	60	32.3	100	60	32.4	100	60	32.4	100	60
11	33.7	60	33.3	99	60	33.5	99	60	33.5	99	60
12	34.3	60	33.8	99	60	34.0	99	60	33.9	99	60
13	34.9	60	34.4	99	60	34.4	99	60	34.6	99	60
17	37.7	60	37.3	99	60	37.5	100	60	37.8	100	60
21	40.5	60	40.1	99	60	40.5	100	60	40.3	100	60
25	41.7	60	41.2	99	60	41.5	100	60	41.4	99	60
29	44.6	60	43.4	97	60	43.5	98	60	43.4	97	60
33	46.2	60	44.7	97	60	45.3	98	60	45.0	97	60
37	48.0	60	47.0	98	60	47.2	98	60	46.8	98	60
41	48.4	60	47.8	99	60	47.9	99	60	47.3	98	60
45	48.9	60	48.5	99	60	48.5	99	59	48.2	99	59
49	49.5	60	49.0	99	60	49.0	99	60	48.8	99	59
53	49.2	60	48.8	99	60	48.5	99	60	48.2	98	59
57[a]	49.9	50	48.7	98	50	49.1	98	50	48.6	97	49
61	50.2	50	49.2	98	50	50.0	100	50	49.3	98	49
65	50.4	50	49.3	98	49	50.0	99	50	49.4	98	49
69	50.5	50	49.7	98	48	50.0	99	50	49.8	99	49
73	51.1	50	50.2	98	48	50.1	98	49	49.8	98	49
77	50.2	50	49.1	98	48	49.2	98	49	49.0	98	49
81	50.0	49	48.7	97	47	49.3	99	48	49.0	98	49
85	50.3	49	49.3	98	47	50.6	101	45	49.6	99	49
89	49.9	49	48.6	97	47	49.8	100	45	49.2	99	48
93	50.6	45	49.7	98	42	50.0	99	44	49.4	98	46
97	50.1	44	49.2	98	42	49.5	99	44	48.7	97	46
101	49.6	42	48.7	98	39	48.8	98	42	48.3	97	45
Mean for weeks											
1-13	30.1		29.8	99		30.0	100		29.9	99	
14-52	45.1		44.3	98		44.5	99		44.3	99	
53-101	50.2		49.2	98		49.6	99		49.1	98	

[a] Interim evaluation occurred during week 53.

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

Weeks on Study	0 ppm		312 ppm			625 ppm			1,250 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	18.5	60	18.5	100	60	18.5	100	60	18.6	101	60
2	20.7	60	20.4	99	60	20.5	99	60	20.3	98	60
3	20.9	60	20.0	96	60	20.4	98	60	19.8	95	60
4	19.6	60	21.4	109	60	20.7	106	60	21.7	111	60
5	21.2	60	20.7	98	60	21.1	100	60	21.6	102	60
6	22.8	60	22.6	99	60	23.1	101	60	22.7	100	60
7	23.6	60	23.5	100	60	23.5	100	60	23.5	100	60
8	23.0	60	23.3	101	60	23.2	101	60	24.1	105	60
9	23.5	60	24.2	103	60	23.4	100	60	24.6	105	60
10	25.7	60	25.4	99	60	25.6	100	60	25.8	100	60
11	25.6	60	25.1	98	60	25.5	100	60	25.9	101	60
12	26.3	60	26.4	100	60	26.4	100	60	26.9	102	60
16	29.9	60	29.9	100	60	30.6	102	59	30.6	102	60
20	31.6	60	32.6	103	60	33.2	105	59	32.6	103	60
24	34.5	60	34.9	101	60	35.1	102	59	36.0	104	60
28	36.8	60	36.9	100	60	37.4	102	59	37.4	102	60
32	39.4	60	39.0	99	60	39.8	101	59	39.2	100	60
36	41.6	60	41.5	100	60	42.2	101	59	41.8	101	60
40	44.7	60	43.6	98	60	44.6	100	59	43.6	98	60
44	45.5	60	45.3	100	60	46.5	102	59	45.2	99	60
48	46.2	60	46.3	100	60	46.7	101	59	45.7	99	60
52	48.5	60	47.2	97	60	48.1	99	59	47.8	99	60
56[a]	49.8	50	48.6	98	50	50.3	101	49	49.3	99	50
60	50.7	50	50.9	100	49	51.4	101	49	51.0	101	50
64	51.6	50	52.3	101	49	52.5	102	49	52.0	101	49
68	53.3	50	54.3	102	48	54.7	103	48	53.8	101	49
72	56.1	49	55.7	99	47	55.9	100	48	54.8	98	49
76	55.6	49	56.0	101	46	55.7	100	48	55.2	99	47
80	56.5	47	56.3	100	45	56.3	100	48	55.6	98	47
84	58.0	47	57.5	99	45	57.3	99	48	56.5	97	47
88	58.7	43	57.5	98	45	58.2	99	48	56.2	96	45
92	57.2	42	57.1	100	44	57.3	100	47	55.5	97	45
96	58.5	41	58.1	99	43	58.1	99	46	55.3	95	43
100	57.7	39	57.0	99	42	56.0	97	44	54.2	94	38
Mean for weeks											
1-13	22.6		22.6	100		22.7	100		23.0	102	
14-52	39.9		39.7	100		40.4	102		40.0	101	
53-100	55.3		55.1	100		55.3	100		54.1	98	

[a] Interim evaluation occurred during week 53.

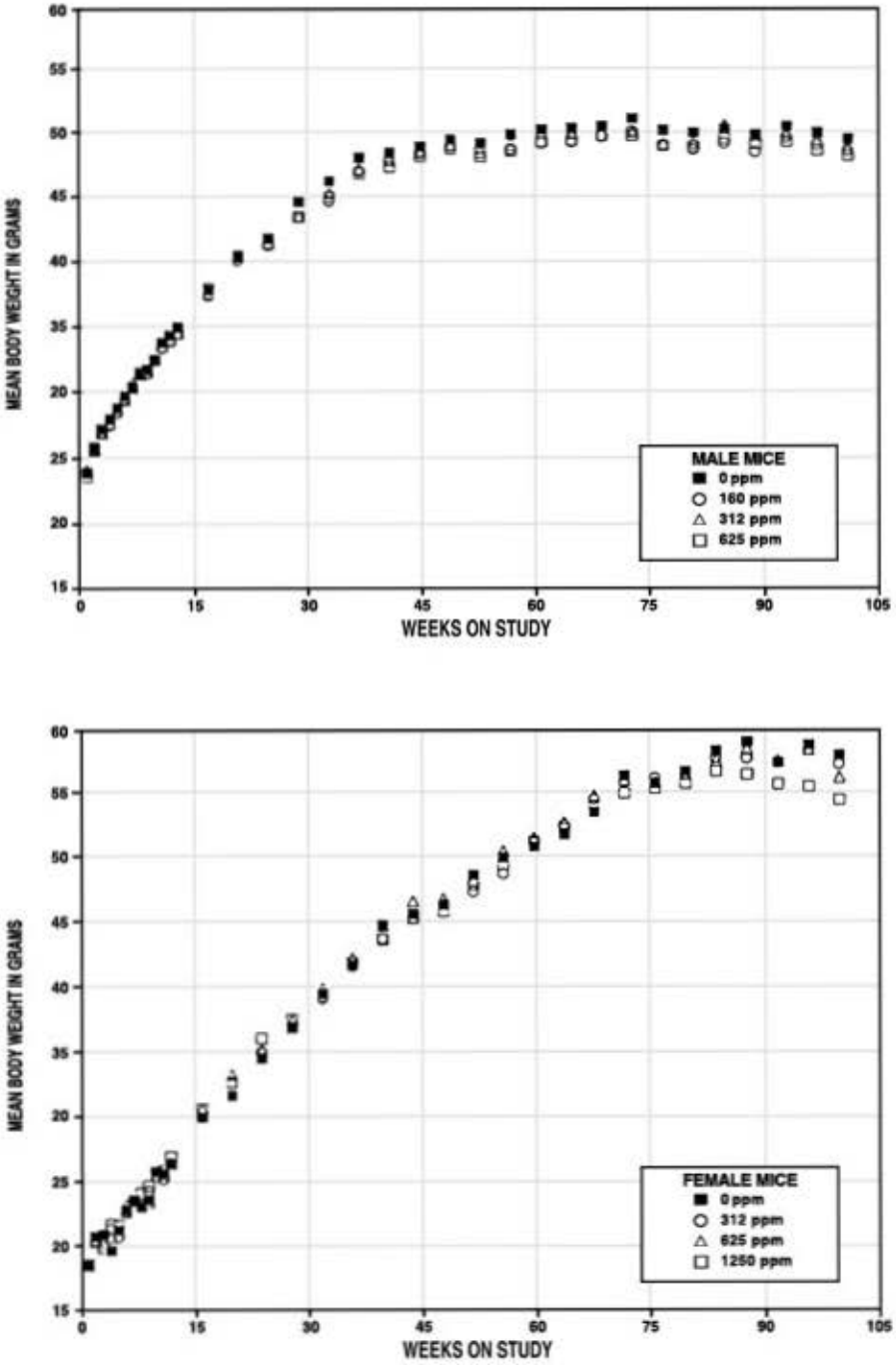


FIGURE 4
Growth Curves for Male and Female Mice Exposed to Emodin in Feed for 2 Years

Pathology and Statistical Analyses

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

Kidney: Although not significantly increased, there were rare renal tubule neoplasms in the kidney of males. The incidence of adenoma or carcinoma (combined) in males exposed to 312 ppm exceeded the historical control range (Tables 17, C1, and C4a). Renal tubule hyperplasia, adenoma, and carcinoma are considered to represent a morphologic and biologic continuum of proliferative lesions; however, there was no significant increase in the incidences of hyperplasia. Initially, a single H&E-stained section of each kidney was prepared and evaluated microscopically. Past experience has shown that microscopic examination of additional sections of the kidney results in identification of additional proliferative lesions and has been helpful in assessing a potential chemical-related effect in the kidney. In this study, additional sections of the remaining formalin-fixed kidneys were taken at 0.5-mm intervals, resulting in approximately four additional sections per animal. A single additional adenoma was identified in a 625 ppm male (Table 17).

Hyperplasia was generally a focal, minimal to moderate lesion consisting of tubules that were dilated two to five times the normal diameter and were lined by increased numbers of tubule epithelial cells that partially or totally filled the tubule lumen. Usually, it was associated with dilatation of the tubule. Cells within the hyperplastic lesions varied slightly in size but otherwise appeared similar to normal tubule epithelial cells. Renal tubule adenomas were larger, discrete lesions, generally ranging from five tubules in diameter up to approximately 1 cm. Cells within adenomas consisted of relatively normal-appearing tubule epithelial cells that sometimes formed solid masses of multiple clusters of cells. Renal tubule carcinomas were larger than adenomas, associated with hemorrhage, necrosis or local invasion, and cellular anaplasia or atypia.

At the 12-month interim evaluation, the severity of nephropathy was slightly increased in males exposed to 625 ppm (Table 17; severity was graded using the criteria discussed for the 14-week rat study). Also at 12 months, the severity of nephropathy increased from minimal to mild in females exposed to 1,250 ppm; the incidence in this group was significantly increased (Tables 17 and D4). At 2 years, the severities of nephropathy were slightly increased in males receiving 625 ppm and females receiving 1,250 ppm. The incidences of nephropathy were significantly increased in all exposed female groups. The increased severities and incidences of nephropathy in the 2-year studies were considered to be exposure related, which was consistent with the treatment-related nephropathy in the 14-week studies and the 12-month interim evaluation.

The nephropathy was focal in distribution, mainly located in the cortical tubules and to a lesser extent involving the glomeruli. The nephropathy observed in the exposed groups was similar to that ordinarily seen as an age-related change in control mice. In the glomeruli and Bowman's capsule, the basement membrane was thickened, while the Bowman's capsule epithelial cells were atrophic or regenerated. Proliferation of fibrous tissue and adhesions between the glomeruli to their capsules were also present. In the tubules, the range of changes included atrophy and regeneration of epithelial cells, thickening of the basement membranes, proliferation of fibrous tissue, the presence of lumenal proteinaceous material and casts, and the presence of lumenal exudate and cysts.

At the 12-month interim evaluation, the incidences of renal tubule pigmentation were significantly increased in all exposed male groups and in females exposed to 625 or 1,250 ppm (Tables 17, C5, and D4). The severities increased with increasing exposure concentration. At 2 years, the incidences of renal tubule pigmentation were significantly increased in all

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
Male				
12-Month Interim Evaluation				
Number Examined Microscopically	10	10	10	10
Nephropathy[a]	10 (1.1)[b]	10 (1.0)	10 (1.0)	10 (1.6)
Renal Tubule Pigmentation	0	7** (1.0)	10** (1.2)	10** (1.9)
2-Year Study				
Single Sections (Standard Evaluation)				
Number Examined Microscopically	49	50	50	50
Nephropathy	49 (1.7)	49 (1.9)	50 (1.8)	49 (2.1)
Renal Tubule Focal Hyperplasia	1 (1.0)	0	0	0
Renal Tubule Pigmentation	0	46** (1.1)	50** (1.4)	50** (2.8)
Renal Tubule Adenoma	0	1	1	0
Renal Tubule Carcinoma	0	0	1	1
Renal Tubule Adenoma or Carcinoma[c]	0	1	2	1
Step Sections (Extended Evaluation)				
Number Examined Microscopically				
Renal Tubule Focal Hyperplasia	1 (3.0)	0	2 (2.5)	1 (2.0)
Renal Tubule Adenoma	0	0	0	1
Single Sections and Step Sections (Combined)				
Number Examined Microscopically				
Renal Tubule Focal Hyperplasia	1 (1.0)	0	2 (2.5)	1 (2.0)
Renal Tubule Adenoma	0	1	1	1
Renal Tubule Carcinoma	0	0	1	1
Renal Tubule Adenoma or Carcinoma	0	1	2	2

	0 ppm	312 ppm	625 ppm	1,250 ppm
Female				
12-Month Interim Evaluation				
Number Examined Microscopically	10	10	10	10
Nephropathy	4 (1.0)	4 (1.0)	5 (1.0)	9* (1.6)
Renal Tubule Pigmentation	0	0	7** (1.0)	10** (1.6)
2-Year Study				
Number Examined Microscopically	49	50	50	49
Nephropathy	22 (1.2)	46** (1.2)	41** (1.2)	48** (1.7)
Renal Tubule Pigmentation	0	37** (1.0)	48** (1.1)	49** (2.3)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (interim evaluations) or the Poly-3 test (2-year study)
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year feed studies with untreated control groups (mean ± standard deviation): 2/851 (0.2% ± 0.7%); range, 0%-2%

exposed groups; severities increased with increasing exposure concentration. The pigment, generally of greater severity in the males, was of a similar nature to that seen in the 14-week studies. The pigment was mainly located in the medulla and corticomedullary junction, in the lumina of the renal tubules, although in some instances pigment granules were present in the tubule epithelium cytoplasm. The presence of pigment usually correlated with the occurrence of nephropathy in the same nephrons, sometimes being localized to the site of the pigment deposition (Plate 4). The pigment stained negatively with PAS stain for identification of carbohydrates, Hall's stain for bile, and Perl's Prussian blue test for identification of iron; therefore, it was presumed to represent deposits of emodin or emodin metabolite(s).

All Organs: A decrease in the incidence of malignant lymphoma was noted in male mice receiving 625 ppm (0 ppm, 5/50; 160 ppm, 3/50; 312 ppm, 3/50; 625 ppm, 1/50; Table C3). This incidence was at the lower end of the historical control range (Table C4b).

Lung: The incidences of alveolar/bronchiolar carcinoma were decreased in groups of exposed male mice (10/50, 9/50, 6/50, 5/50; Table C3). This apparent decrease was primarily due to the high incidences in the control and 160 ppm groups; the incidences in these groups were outside the current historical control range [54/852 (6.4% ± 3.8%); range, 2%-14%]. The incidences of alveolar epithelial hyperplasia were not decreased (2/50, 1/50, 4/50, 0/50; Table C5), and no decreases were observed in female mice. This marginal decrease in the incidences of alveolar/bronchiolar carcinoma was not considered to be treatment related.

Genetic Toxicology

Emodin, tested in two separate studies in a preincubation assay, was mutagenic in *Salmonella typhimurium* strain TA100 in the presence of induced rat or hamster S9 liver enzymes over a concentration range of 1 to 666 μg/plate (Table E1). No mutagenicity was detected with emodin in this assay in strain TA98, with or without S9. Chromosomal aberrations were induced in cultured Chinese hamster ovary cells treated with 10 to 20 μg/mL emodin in the absence of S9 activation and with 100 to 200 μg/mL emodin in the presence of S9 (Table E2); the response observed without S9 was stronger than with S9. Three separate *in vivo* micronucleus tests were performed with emodin in attempts to clarify a complicated response pattern; most of the tests gave negative results. Emodin was tested for induction of micronuclei in polychromatic erythrocytes in standard three-exposure studies; bone marrow was analyzed 24 hours after the third injection, and results in male rats and male and female mice were negative (Table E3). Peripheral blood samples from the same mice at the end of the 72-hour exposure period were also analyzed for frequency of micronuclei, and a statistically positive response was obtained for male mice only. Considering both the bone marrow and the peripheral blood data, the three-exposure micronucleus test was judged to be negative overall in male and female mice. In peripheral blood samples from mice in the 14-week feed study, an increase in the frequency of micronucleated normochromatic erythrocytes was seen in females, but not in males (Table E4). The small increase in normochromatic erythrocytes observed in the female mice was statistically significant ($P=0.001$), but no individual exposed group value differed significantly from the control value; the result in female mice was concluded to be weakly positive.

DISCUSSION AND CONCLUSIONS

Emodin is a naturally occurring anthraquinone present in vegetable laxatives containing extracts of the dried roots and/or bark of several plants of the genus *Rhamnus*. Reports by Mori *et al.* (1985, 1986) that 1,8-dihydroxyanthraquinone, a commonly used laxative ingredient, caused tumors in the gastrointestinal tract of rats raised the possibility of an association between colorectal cancer and the use of laxatives containing anthraquinones. Because emodin is a hydroxyanthraquinone structurally similar to 1,8-dihydroxyanthraquinone, it was considered a potential carcinogen and selected for in-depth evaluation.

During the 16-day and 14-week studies, exposure to emodin was associated with a number of lesions in the kidney of rats and mice. In the 14-week rat study, hyaline droplets characterized by the presence of multiple hyalinized, intensely stained, red-orange oval structures, sometimes surrounded by a pale halo, were present in the cytoplasm of cortical tubule epithelial cells in all exposed groups of male rats and female rats exposed to 312.5, 625, or 1,250 ppm. No droplets were present in the kidneys of female rats exposed to 2,500 or 5,000 ppm. If the droplets contained protein, then their absence may be related to the significant reduction in total serum protein and albumin concentrations observed in females exposed to 2,500 or 5,000 ppm at the end of the 14-week study. A similar decrease was not noted in males.

Morphologically, the droplets were different than the α2u-globulin-associated hyaline droplets observed in control male rats. In males, the severities of the droplets exhibited an unusual dose response with the greatest severity in the 1,250 ppm group; the severities in the 312.5 and 625 ppm groups were greater than in the 2,500 or 5,000 ppm groups. In an effort to determine whether the presence of droplets was associated with an increased proliferative response, kidney sections from three control males, three male rats exposed to 1,250 ppm, and three males exposed to 5,000 ppm were stained immunohistochemically for proliferating cell nuclear antigen. Mean labeling indexes were similar in all groups. The presence of droplets was not considered a major dose-limiting toxic response in rats.

In the 14-week mouse study, renal tubule pigmentation was present in all exposed groups of males but only in females exposed to 1,250 ppm or greater. In males and females, both the incidences and severities generally increased with increasing exposure concentration. Although the pigment was not positively identified, it was considered to be emodin or possibly a metabolite. In male and female mice, the pigment was located in the lumen of the tubules. Because the incidences and severities of nephropathy generally increased with increasing exposure concentration and appeared to parallel pigmentation, the pigmentation may have obstructed the tubules, thus exacerbating the nephropathy. Because the incidences and severities of pigmentation and nephropathy in male mice were minimal at 625 ppm but increased markedly at 1,250 ppm, 625 ppm was selected as the highest exposure concentration for the 2-year study in male mice. In female mice, pigmentation and nephropathy were present only in groups exposed to 1,250 ppm or greater. The incidences and severities were increased markedly at 5,000 ppm but were similar in the 1,250 and 2,500 ppm groups. Therefore, selecting 1,250 ppm as the highest exposure concentration for the 2-year study in female mice provided an adequate challenge while avoiding the additional potential for toxicity that might accompany the use of 2,500 ppm.

Exposure to emodin for 2 years did not reduce the survival of any groups of rats or mice and caused relatively modest reductions in the mean body weights of rats during the study. However, unlike many other anthraquinones evaluated by the NTP, emodin was not unequivocally carcinogenic.

Zymbal's gland carcinomas were present in three female rats exposed to 2,500 ppm. The Zymbal's glands, specialized sebaceous glands about 3 to 5 mm in diameter lying anterioventral to the orifices of the external ears, were examined microscopically when observed to be grossly abnormal or enlarged at

necropsy. Zymbal's gland carcinomas generally occur late in life, are relatively fast growing and highly invasive, and produce body weight loss and debilitation. Zymbal's gland neoplasms seldom occur spontaneously but are readily induced by a variety of carcinogens. To date, treatment-related increased incidences of Zymbal's gland neoplasms have occurred in male and/or female rats in studies of 21 chemicals tested by the NTP (1998). Of these chemicals, 19 were mutagenic in *Salmonella*, and 20 of 21 were multisite carcinogens. In only one of the 21 studies did the increase occur in females and not males. Many of these chemicals also caused neoplasms of other specialized sebaceous glands (preputial or clitoral gland) and/or the skin. An exception is pentaerythritol tetranitrate (NTP, 1989). In that NTP feed study, the only potential tumorigenic findings (equivocal evidence of carcinogenicity) were low incidences of Zymbal's gland neoplasms [adenoma or carcinoma (combined)] in male (0 ppm, 0/49; 25,000 ppm, 3/45; 50,000 ppm, 2/41) and female (0 ppm, 0/36; 6,200 ppm, 1/37; 12,500 ppm, 3/35) rats. Although emodin is not a strong genotoxin, it is extensively metabolized in the liver (Bachmann and Schlatter, 1981) and has at least one probable metabolite, 2-hydroxyemodin, which is mutagenic in *Salmonella* (Masuda and Ueno, 1984). Emodin clearly is not a multisite carcinogen, and the pattern of tumorigenic response in this study was dissimilar to that observed for all previously studied chemicals that clearly produced an effect on the Zymbal's gland. Additionally, there was an absence of a similar response in male rats. However, the incidence of Zymbal's gland adenoma exceeded the range observed for current historical controls. Therefore, the occurrence of three Zymbal's gland carcinomas in female rats exposed to 2,500 ppm was considered an equivocal finding.

Renal tubule adenomas and carcinomas were present in exposed male mice, including one adenoma each in the 160 and 312 ppm groups and one carcinoma each in the 312 and 625 ppm groups. Renal tubule neoplasms are rare in male mice; the historical control incidence is 2/851 (0.2% ± 0.7%; range, 0%-2%). The presence of these neoplasms suggests an association with emodin exposure. Hyperplasia, adenoma, and carcinoma of the renal tubule are considered to represent a morphologic and biologic continuum of proliferative lesions; however, there was no significant increase in the incidences of renal tubule hyperplasia in male mice. Past experience has shown that microscopic examination of additional sections of the kidney may result in identification of additional proliferative lesions and has been helpful in assessing a potential chemical related effect in the kidney. In this study, additional kidney sections revealed the presence of a single additional adenoma in a male 625 ppm mouse. Therefore, based on the absence of an increase in the incidence of renal tubule hyperplasia and the discovery of only one additional adenoma in the extended evaluation, the low incidence of renal tubule neoplasms in exposed male mice was considered an uncertain finding.

Danthron (1,8-dihydroxyanthraquinone), a hydroxyanthraquinone structurally similar to emodin, administered in feed caused tumors in the gastrointestinal tract of ACI rats and the livers of CH3/HeN mice (Mori *et al.*, 1985, 1986). Exposure of ACI rats to 1-hydroxyanthraquinone in feed caused tumors in the gastrointestinal tract and liver (Mori *et al.*, 1990). However, there was no indication that exposure to emodin had any effect on the gastrointestinal tract of rats or mice in either the 16-day or 14-week studies, in which exposure concentrations were up to 50,000 ppm, or in the 2-year studies. Although emodin is extensively metabolized in the liver, there was no evidence of any carcinogenic response in the liver of rats or mice. Serum albumin concentrations were decreased for female rats exposed to 2,500 or 5,000 ppm in the 14-week study; however, there were no other indications of hepatotoxicity. Instead, it appears that emodin was effectively detoxified by the liver.

In most previous NTP studies in which chemical exposure reduced the incidences of mononuclear cell leukemia, there was an accompanying treatment-associated toxic response in the spleen during the prechronic studies (Elwell *et al.*, 1996). Also, splenectomy of F344/N rats aged 1 to 2 months markedly reduced the incidence of mononuclear cell leukemia. In the present study, however, exposure to emodin had no effect on the spleen or hematopoietic system in rats.

Although little is known about the molecular details underlying the development of mononuclear cell leukemia in F344/N rats or malignant lymphoma in $B6C3F_1$ mice, emodin has been shown to be an inhibitor of several protein kinases involved in signal transduction. These protein kinases included protein tyrosine kinase $p56^{lck}$ (Jayasuriya *et al.*, 1992), phosphatidylinositol-3-kinase, protein kinase C, and c-src (Frew *et al.*, 1994). In human breast cancer cells overexpressing the *HER-2/neu* proto-oncogene, which encodes a transmembrane tyrosine kinase growth factor receptor, emodin inhibited the tyrosine kinase activity of the $p185^{neu}$ gene product, preferentially blocked proliferation of these cells, and induced their differentiation into mature breast cells (Zhang *et al.*, 1995; Zhang and Hung, 1996). Emodin also selectively blocked the growth of *v-ras* transformed bronchial epithelial cells (Chan *et al.*, 1993). In view of the important role of protein tyrosine phosphorylation in many fundamental cellular processes and, in particular, its role in the regulation of hematopoiesis (Ihle *et al.*, 1994), it is plausible that inhibition or alteration of tyrosine phosphorylation by emodin may be involved in reducing the incidences of mononuclear cell leukemia in rats and malignant lymphoma in male mice.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of emodin in male F344/N rats exposed to 280, 830, or 2,500 ppm. There was *equivocal evidence of carcinogenic activity* of emodin in female F344/N rats based on a marginal increase in the incidence of Zymbal's gland carcinoma. There was *equivocal evidence of carcinogenic activity* of emodin in male $B6C3F_1$ mice based on a low incidence of uncommon renal tubule neoplasms. There was *no evidence of carcinogenic activity* of emodin in female $B6C3F_1$ mice exposed to 312, 625, or 1,250 ppm.

Exposure of rats to emodin resulted in increased incidences of renal tubule hyaline droplets and pigmentation in males, increased incidences of renal tubule hyaline droplets in females, and increased severities of renal tubule pigmentation in males and females. Emodin exposure resulted in increased incidences of renal tubule pigmentation in male and female mice and increased incidences of nephropathy in female mice.

Incidences of mononuclear cell leukemia decreased in male and female rats exposed to 2,500 ppm.

---

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

REFERENCES

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Bachmann, M., and Schlatter, C.H. (1981). Metabolism of [^{14}C] emodin in the rat. *Xenobiotica* **11**, 217-225.

Bachmann, M., Lüthy, J., and Schlatter, C. (1979). Toxicity and mutagenicity of molds of the *Aspergillus glaucus* group. Identification of physcion and three related anthraquinones as main toxic constituents from *Aspergillus chevalieri*. *J. Agric. Food Chem.* **27**, 1342-1347.

Bailer, A.J., and Portier, C.J. (1988). Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* **44**, 417-431.

Bieler, G.S., and Williams, R.L. (1993). Ratio of estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics* **49**, 793-801.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Brown, J.P., and Brown, R.J. (1976). Mutagenesis by 9,10-anthraquinone derivatives and related compounds in *Salmonella typhimurium*. *Mutat. Res.* **40**, 203-224.

Bruggeman, I.M., and van der Hoeven, J.C.M. (1984). Lack of activity of the bacterial mutagen emodin in HGPRT and SCE assay with V79 Chinese hamster cells. *Mutat. Res.* **138**, 219-224.

Brunton, L.L. (1985). Laxatives. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 7th ed. (A.G. Gilman, L.S. Goodman, T.W. Rall, and F. Murad, Eds.), pp. 994-1003. Macmillan Publishing Company, New York.

Chan, T.C., Chang, C., Koonchanok, N.M., and Geahlen, R.L. (1993). Selective inhibition of the growth of RAS-transformed human bronchial epithelial cells by emodin, a protein-tyrosine kinase inhibitor. *Biochem. Biophys. Res. Comm.* **193**, 1152-1158.

Cheh, A.M., Hooper, A.B., Skochdopole, J., Henke, C.A., and McKinnell, R.G. (1980). A comparison of the ability of frog and rat S-9 to activate promutagens in the Ames test. *Environ. Mutagen.* **2**, 487-508.

Code of Federal Regulations (CFR) **21**, Part 58.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology. Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co., Inc., Princeton, NJ.

Dixon, W.J., and Massey, F.J., Jr. (1951). *Introduction to Statistical Analysis*, 1st ed., pp. 145-147. McGraw-Hill Book Company, Inc., New York.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Elwell, M.R., Dunnick, J.K., and Haseman, J.K. (1996). Chemicals associated with decreases in the incidence of mononuclear cell leukemia in the Fischer rat. *Toxicol. Pathol.* **24**, 238-245.

Everhart, J.E., Go, V.L., Johannes, S., Fitzsimmons, S.C., Roth, H.P., and White, L.R. (1989). A longitudinal survey of self-reported bowel habits in the United States. *Dig. Dis. Sci.* **34**, 1153-1162.

Fluck, E.R., Poirier, L.A., and Ruelius, H.W. (1976). Evaluation of a DNA polymerase-deficient mutant of *E. coli* for the rapid detection of carcinogens. *Chem. Biol. Interact.* **15**, 219-231.

Frew, T., Powis, G., Berggren, M., Abraham, R.T., Ashendel, C., Zalkow, L.H., Hudson, C., Quazia, S., Gruszecka-Kowalik, E., Merriman, R., and Bonjouklian, R. (1994). A multiwell assay for inhibitors of phosphatidylinositol-3-kinase and the identification of natural product inhibitors. *Anticancer Res.* **14**, 2425-2428.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* **10** (Suppl. 10), 1-175.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCI* **62**, 957-974.

Gibson, D.P., Brauninger, R., Shaffi, H.S., Kerckaert, G.A., LeBoeuf, R.A., Isfort, R.J., and Aardema, M.J. (1997). Induction of micronuclei in Syrian hamster embryo cells: Comparison to results in the SHE cell transformation assay for National Toxicology Program test chemicals. *Mutat. Res.* **392**, 61-70.

Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods*, pp. 120-123. John Wiley and Sons, New York.

Ihle, J.N., Witthuhn, B.A., Silvennoinen, Q.O., Tang, B., and Yi, T. (1994). Protein tyrosine phosphorylation in the regulation of hematopoiesis by receptors of the cytokine-receptor superfamily. *Blood Cells* **20**, 65-82.

Integrated Laboratory Systems (ILS) (1990). Micronucleus Data Management and Statistical Analysis Software, Version 1.4. ILS, P.O. Box 13501, Research Triangle Park, NC 27707.

Jayasuriya, H., Koonchanok, N.M., Geahlen, R.L., McLaughlin, J.L., and Chang, C. (1992). Emodin, a protein tyrosine kinase inhibitor from *Polygonum cuspidatum*. *J. Nat. Prod.* **55**, 696-698.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Kerckaert, G.A., Isfort, R.J., Carr, G.J., Aardema, M.J., LeBoeuf, R.A. (1996). A comprehensive protocol for conducting the Syrian hamster embryo cell transformation assay at pH 6.70. *Mutat. Res.* **356**, 65-84.

Krivobok, S., Seigle-Murandl, F., Steiman, R., Marzin, D.R., and Betina, V. (1992). Mutagenicity of substituted anthraquinones in the Ames/Salmonella microsome system. *Mutat. Res.* **279**, 1-8.

Liberman, D.F., Schaefer, F.L., Fink, R.C., Ramgopal, M., Ghosh, A.C., and Mulcahy, R. (1980). Mutagenicity of islandicin and chrysophanol in the Salmonella/microsome system. *Appl. Environ. Microbiol.* **40**, 476-479.

Lide, D.R., Ed. (1998). *CRC Handbook of Chemistry and Physics*, 79th ed., p. 3-14. CRC Press, Boca Raton, FL.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Masuda, T., and Ueno, Y. (1984). Microsomal transformation of emodin into a direct mutagen. *Mutat. Res.* **125**, 135-144.

Masuda, T., Haraikawa, K., Morooka, N., Nakano, S., and Ueno, Y. (1985). 2-Hydroxyemodin, an active metabolite of emodin in the hepatic microsomes of rats. *Mutat. Res.* **149**, 327-332.

Mengs, U., Krumbiegel, G., and Völkner, W. (1997). Lack of emodin genotoxicity in the mouse micronucleus assay. *Mutat. Res.* **393**, 289-293.

The Merck Index (1989). 11th ed. (S. Budavari, Ed.), p. 557. Merck and Company, Rahway, NJ.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Mori, H., Kawai, K., Ohbayashi, F., Kuniyasu, T., Yamazaki, M., Hamasaki, T., and Williams, G.M. (1984). Genotoxicity of a variety of mycotoxins in the hepatocyte primary culture/DNA repair test using rat and mouse hepatocytes. *Cancer Res.* **44**, 2918-2923.

Mori, H., Sugie, S., Niwa, K., Takahashi, M., and Kawai, K. (1985). Induction of intestinal tumors in rats by chrysazin. *Br. J. Cancer* **52**, 781-783.

Mori, H., Sugie, S., Niwa, K., Yoshimi, N., Tanaka, T., and Hirono, I. (1986). Carcinogenicity of chrysazin in large intestine and liver of mice. *Jpn. J. Cancer Res.* **77**, 871-876.

Mori, H., Yoshimi, N., Iwata, H., Mori, Y., Hara, A., Tanaka, T., and Kawai, K. (1990). Carcinogenicity of naturally occurring 1-hydroxyanthraquinone in rats: Induction of large bowel, liver, and stomach neoplasms. *Carcinogenesis* **11**, 799-802.

Morrison, D.F. (1976). *Multivariate Statistical Methods*, 2nd ed., pp. 170-179. McGraw-Hill Book Company, New York.

Mueller, S.O., Stopper, H., and Dekant, W. (1998a). Biotransformation of the anthraquinones emodin and chrysophanol by cytochrome P450 enzymes: Bioactivation to genotoxic metabolites. *Drug Metab. Dispos.* **26**, 540-546.

Mueller, S.O., Lutz, W.K., and Stopper, H. (1998b). Factors affecting the genotoxic potency ranking of natural anthraquinones in mammalian cell culture systems. *Mutat. Res.* **414**, 125-129.

Müller, S.O., Eckert, I., Lutz, W.K., and Stopper, H. (1996). Genotoxicity of the laxative drug components emodin, aloe-emodin and danthron in mammalian cells: Topoisomerase II mediated? *Mutat. Res.* **371**, 165-173.

Murakami, H., Kobayashi, J., Masuda, T., Morooka, N., and Ueno, Y. (1987). ω-Hydroxyemodin, a major hepatic metabolite of emodin in various animals and its mutagenic activity. *Mutat. Res.* **180**, 147-153.

National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1978a). Bioassay of 2-Aminoanthraquinone for Possible Carcinogenicity (CAS No. 117-79-3). Technical Report Series No. 144. NIH Publication No. 78-1399. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1978b). Bioassay of 1-Amino-2-methylanthraquinone for Possible Carcinogenicity (CAS No. 82-28-0). Technical Report Series No. 111. NIH Publication No. 78-1366. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1978c). Bioassay of 2-Methyl-1-nitroanthraquinone for Possible Carcinogenicity (CAS No. 129-15-7). Technical Report Series No. 29. NIH Publication No. 78-829. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Institutes of Health (NIH) (1978). Open Formula Rat and Mouse Ration (NIH-07). Specification NIH-11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, NIH, Bethesda, MD.

National Toxicology Program (NTP) (1986). Toxicology and Carcinogenesis Studies of C.I. Disperse Blue 1 (CAS No. 2475-45-8) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 299. NIH Publication No. 86-2555. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1987). National Toxicology Program (NTP) General Statement of Work for the Conduct of Toxicity and Carcinogenicity Studies in Laboratory Animals. Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and Mice, 10/31/82 version (updated April 1987). Research Triangle Park, NC.

National Toxicology Program (NTP) (1989). Toxicology and Carcinogenesis Studies of Pentaerythritol Tetranitrate (CAS No. 78-11-5) with 80% *d*-Lactose Monohydrate (PETN, NF) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 365. NIH Publication No. 89-2820. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

National Toxicology Program (NTP) (1996). Toxicology and Carcinogenesis Studies of 1-Amino-2,4-dibromoanthraquinone (CAS No. 81-49-2) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 383. NIH Publication No. 96-2838. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1998). Tumor Incidence in Control Animals by Route and Vehicle of Administration in F344/N Rats. Analytical Sciences, Inc., Durham, NC.

Nusko, G., Schneider, B., Muller, G., Kusche, J., and Hahn, E.G. (1993). Retrospective study on laxative use and melanosis coli as risk factors for colorectal neoplasma. *Pharmacology* **47** (Suppl. 1), 234-241.

Nyska, A., Maronpot, R.R., Eldridge, S.R., Haseman, J.K., and Hailey, J.R. (1997). Alteration in cell kinetics in control B6C3F$_1$ mice infected with *Helicobacter hepaticus*. *Toxicol. Pathol.* **25**, 591-596.

Piegorsch, W.W., and Bailer, A.J. (1997). *Statistics for Environmental Biology and Toxicology*, Section 6.3.2. Chapman and Hall, London.

Portier, C.J., and Bailer, A.J. (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam. Appl. Toxicol.* **12**, 731-737.

Portier, C.J., Hedges, J.C., and Hoel, D.G. (1986). Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. *Cancer Res.* **46**, 4372-4378.

Sadtler Standard Spectra. IR No. 3600. Sadtler Research Laboratories, Philadelphia.

Seely, J.C. (1991). Carcinoma of the auditory sebaceous glands, rat. In *Eye and Ear. Monographs on Pathology of Laboratory Animals* (T.C. Jones, U. Mohr, and R.D. Hunt, Eds.), pp 145-149. Springer-Verlag, Berlin.

Shelby, M.D. (1988). The genetic toxicity of human carcinogens and its implications. *Mutat. Res.* **204**, 3-15.

Shelby, M.D., and Witt, K.L. (1995). Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ. Mol. Mutagen.* **25**, 302-313.

Shelby, M.D., and Zeiger, E. (1990). Activity of human carcinogens in the Salmonella and rodent bone-marrow cytogenetics tests. *Mutat. Res.* **234**, 257-261.

Shelby, M.D., Erexson, G.L., Hook, G.J., and Tice, R.R. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environ. Mol. Mutagen.* **21**, 160-179.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* **67**, 233-241.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. *Science* **236**, 933-941.

Tikkanen, L., Matsushima, T., and Natori, S. (1983). Mutagenicity in anthraquinones in the Salmonella preincubation test. *Mutat. Res.* **116**, 297-304.

Ueno, Y. (1984). Hydroxyanthraquinones (skyrines). In *Mycotoxins - Production, Isolation and Purification* (V. Betina, Ed.), pp. 329-350. Elsevier, Amsterdam.

Ueno, Y., and Kubota, K. (1976). DNA-attacking ability of carcinogenic mycotoxins in recombination-deficient mutant cells of Bacillus subtilis. *Cancer Res.* **36**, 445-451.

Watanabe, K., Sakamoto, K., and Sasaki, T. (1998). Comparisons on chemically-induced mutation among four bacterial strains, *Salmonella typhimurium* TA102 and TA2638, and *Escherichia coli* WP2/pKM101 and WP2 *uvr*A/pKM101: Collaborative study II. *Mutat. Res.* **412**, 17-31.

Wehner, A.P., Stuart, B.O., and Sanders, C.L. (1979). Inhalation studies with Syrian golden hamsters. *Prog. Exp. Tumor Res.* **24**, 177-198.

Westendorf, J., Marquardt, H., Poginsky, B., Dominiak, M., Schmidt, J., and Marquardt, H. (1990). Genotoxicity of naturally occurring hydroxyanthraquinones. *Mutat. Res.* **240**, 1-12.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Witt, K.L., Knapton, A., Wehr, C.M., Hook, G.J., Mirsalis, J., Shelby, M.D., and MacGregor, J.T. (2000). Micronucleated erythrocyte frequency in peripheral blood of $B6C3F_1$ mice from short-term, prechronic, and chronic studies of the NTP Carcinogenesis Bioassay Program. *Environ. Mol. Mutagen.* **36,** 163-194.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **19** (Suppl. 21), 2-141.

Zhang, L., and Hung, M.C. (1996). Sensitization of *HER-2/neu*-overexpressing non-small lung cancer cells to chemotherapeutic drugs by tyrosine kinase inhibitor emodin. *Oncogene* **12**, 571-576.

Zhang, L., Chang, C., Bacus, S.S., and Hung, M.C. (1995). Suppressed transformation and induced differentiation of *HER-2/neu* overexpressing breast cancer cells by emodin. *Cancer Res.* **55**, 3890-3896.

APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF EMODIN

TABLE A1 Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Emodin 70
TABLE A2 Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin 74
TABLE A3 Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Emodin 92
TABLE A4a Historical Incidence of Nasal Squamous Cell Carcinoma in Untreated Male F344/N Rats 96
TABLE A4b Historical Incidence of Mononuclear Cell Leukemia in Untreated Male F344/N Rats 96
TABLE A5 Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Emodin 97

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

	0 ppm	280 ppm	830 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	65	65	65	65
6-Month interim evaluation	10	10	10	10
12-Month interim evaluation	5	5	5	5
Early deaths				
Moribund	17	26	27	16
Natural deaths	3	3	2	4
Survivors				
Terminal sacrifice	30	21	21	30
Animals examined microscopically	65	65	65	65
Systems Examined at 6 and 12 Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Intestine large, cecum	(49)	(50)	(50)	(49)
Intestine small, duodenum	(48)	(50)	(49)	(50)
Carcinoma	1 (2%)			
Intestine small, jejunum	(49)	(48)	(50)	(49)
Schwannoma malignant, metastatic, skin				1 (2%)
Intestine small, ileum	(50)	(50)	(48)	(49)
Sarcoma				1 (2%)
Liver	(50)	(50)	(50)	(50)
Cholangiocarcinoma				1 (2%)
Fibrosarcoma, metastatic, spleen			1 (2%)	
Hepatocellular carcinoma		3 (6%)	3 (6%)	
Hepatocellular adenoma	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Mesentery	(8)	(7)	(13)	(15)
Sarcoma				2 (13%)
Pancreas	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, spleen			1 (2%)	
Mixed tumor benign	2 (4%)			
Salivary glands	(50)	(50)	(50)	(49)
Schwannoma malignant			1 (2%)	
Stomach, forestomach	(50)	(50)	(49)	(48)
Squamous cell papilloma				1 (2%)
Stomach, glandular	(50)	(50)	(49)	(48)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant, metastatic, skin				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Adrenal medulla	(50)	(49)	(50)	(50)
Pheochromocytoma malignant	1 (2%)		1 (2%)	1 (2%)
Pheochromocytoma benign	6 (12%)	11 (22%)	5 (10%)	9 (18%)
Bilateral, pheochromocytoma benign	1 (2%)	2 (4%)	1 (2%)	
Islets, pancreatic	(50)	(50)	(49)	(50)
Adenoma	2 (4%)	5 (10%)	1 (2%)	1 (2%)
Carcinoma	3 (6%)	1 (2%)	1 (2%)	
Parathyroid gland	(48)	(45)	(45)	(47)
Adenoma			1 (2%)	
Pituitary gland	(49)	(50)	(48)	(50)
Pars distalis, adenoma	15 (31%)	9 (18%)	10 (21%)	13 (26%)
Pars intermedia, adenoma		1 (2%)	1 (2%)	1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	3 (6%)	5 (10%)	3 (6%)	3 (6%)
C-cell, carcinoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Follicular cell, adenoma	1 (2%)	1 (2%)		
Follicular cell, carcinoma		2 (4%)	2 (4%)	
General Body System				
Peritoneum	(2)	(1)	(3)	(1)
Genital System				
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	5 (10%)	1 (2%)	2 (4%)	3 (6%)
Carcinoma	3 (6%)	2 (4%)	1 (2%)	3 (6%)
Prostate	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		1 (2%)	3 (6%)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	34 (68%)	30 (60%)	37 (74%)	35 (70%)
Interstitial cell, adenoma	7 (14%)	13 (26%)	7 (14%)	13 (26%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Schwannoma malignant, metastatic, skin				1 (2%)
Lymph node	(12)	(21)	(18)	(11)
Lymph node, mandibular	(49)	(50)	(48)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Fibrosarcoma			1 (2%)	
Schwannoma malignant, metastatic, skin				1 (2%)
Thymus	(46)	(47)	(48)	(48)
Thymoma malignant	1 (2%)			

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Integumentary System				
Mammary gland	(49)	(47)	(49)	(49)
Fibroadenoma	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Skin	(50)	(50)	(49)	(49)
Basal cell adenoma		1 (2%)		
Basal cell carcinoma		1 (2%)		1 (2%)
Keratoacanthoma	3 (6%)	3 (6%)	3 (6%)	3 (6%)
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma		1 (2%)		2 (4%)
Trichoepithelioma	1 (2%)			1 (2%)
Sebaceous gland, adenoma	1 (2%)	1 (2%)		
Subcutaneous tissue, fibroma	1 (2%)	3 (6%)	2 (4%)	5 (10%)
Subcutaneous tissue, fibrosarcoma		1 (2%)		
Subcutaneous tissue, hemangiopericytoma	1 (2%)			
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Subcutaneous tissue, histiocytic sarcoma			1 (2%)	
Subcutaneous tissue, lipoma	1 (2%)	1 (2%)	1 (2%)	
Subcutaneous tissue, sarcoma	1 (2%)			
Subcutaneous tissue, schwannoma malignant				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma			1 (2%)	1 (2%)
Skeletal muscle	(1)			(2)
Schwannoma malignant, metastatic, skin				1 (50%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant	2 (4%)	1 (2%)		
Oligodendroglioma malignant		1 (2%)		
Pineal gland, meningioma malignant		1 (2%)		
Spinal cord	(2)	(5)	(8)	(3)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma		1 (2%)		
Histiocytic sarcoma		1 (2%)	1 (2%)	
Osteosarcoma, metastatic, bone				1 (2%)
Schwannoma malignant, metastatic, skin				1 (2%)
Nose	(50)	(50)	(50)	(50)
Squamous cell carcinoma				2 (4%)
Special Senses System				
Zymbal's gland		(1)		(1)
Carcinoma		1 (100%)		1 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, stromal nephroma		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Leukemia mononuclear	28 (56%)	31 (62%)	29 (58%)	18 (36%)
Mesothelioma malignant	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]				
2-Year study	49	50	50	50
Total primary neoplasms				
2-Year study	131	141	124	133
Total animals with benign neoplasms				
2-Year study	45	46	47	49
Total benign neoplasms				
2-Year study	87	91	79	98
Total animals with malignant neoplasms				
2-Year study	36	38	36	28
Total malignant neoplasms				
2-year study	44	50	45	35
Total animals with metastatic neoplasms				
2-Year study			1	2
Total metastatic neoplasms				
2-Year study			2	7

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	122	216	284	406	472	528	590	590	597	599	604	617	617	646	679	686	700	704	711	725	729	729	729	729	729
Carcass ID Number	014	038	010	008	017	032	015	030	027	013	049	026	060	001	044	006	023	051	041	021	002	003	016	018	022
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery						+								+	+		+		+						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mixed tumor benign						X																			
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel				+																					
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant														X											
Pheochromocytoma benign																X									
Bilateral, pheochromocytoma benign																							X		
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Pars distalis, adenoma											X		X	X				X		X					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																								X	X
C-cell, carcinoma																			X						
Follicular cell, adenoma																									
General Body System																									
Peritoneum											+				+										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X	X														X					
Carcinoma							X														X		X		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma						X	X		X	X					X	X	X			X	X	X	X	X	X
Interstitial cell, adenoma												X	X					X	X						

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	024	035	036	040	042	046	047	052	053	054	058	005	007	011	019	020	025	028	029	033	037	043	050	055	056	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma					X																					1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																	X									1
Mesentery			+																	+		+				8
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mixed tumor benign			X																							2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign					X	X		X													X	X				6
Bilateral, pheochromocytoma benign																										1
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X																		X					2
Carcinoma					X	X													X							3
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X						X	X	X		X		X			X		X					X		X	15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma														X												3
C-cell, carcinoma																										1
Follicular cell, adenoma						X																				1
General Body System																										
Peritoneum																										2
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma						X												X								5
Carcinoma																										3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma											X															1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X		X	X	X	X	X	X		X	X	X	X		X	X		X	X	X	X	X	X	34
Interstitial cell, adenoma				X							X								X							7

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	122	216	284	406	472	528	590	590	597	599	604	617	617	646	679	686	700	704	711	725	729	729	729	729	729
Carcass ID Number	014	038	010	008	017	032	015	030	027	013	049	026	060	001	044	006	023	051	041	021	002	003	016	018	022
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+	+	+	+		+	+		+				+	+			+						+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+
Thymoma malignant																						X			
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																		X							
Trichoepithelioma																									
Sebaceous gland, adenoma																									X
Subcutaneous tissue, fibroma																		X							
Subcutaneous tissue, hemangiopericytoma												X													
Subcutaneous tissue, hemangiosarcoma				X																					
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, sarcoma	X																								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									
Peripheral nerve						+												M							
Spinal cord						+												+							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear			+																						
Eye															+							+			
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X		X	X		X	X	X	X				X	X	X		X	X	X		X		X
Mesothelioma malignant											X				X										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	024	035	036	040	042	046	047	052	053	054	058	005	007	011	019	020	025	028	029	033	037	043	050	055	056	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node								+																		12
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Thymoma malignant																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibroadenoma	X																									1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																X							X			3
Trichoepithelioma																								X		1
Sebaceous gland, adenoma																										1
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, hemangiopericytoma																										1
Subcutaneous tissue, hemangiosarcoma																										1
Subcutaneous tissue, lipoma																			X							1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant									X				X													2
Peripheral nerve																										1
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Eye														+												3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X		X	X	X		X				X		X		X	X				X			X	X	28
Mesothelioma malignant																										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 280 ppm

Number of Days on Study	462	464	491	506	511	555	557	561	584	596	610	610	617	630	631	632	636	638	665	673	679	679	679	690	704
Carcass ID Number	1011	1012	0701	1004	1017	1015	0072	0066	0097	0063	0062	0069	0079	0091	0092	0061	1019	0087	1101	1016	0064	0075	0078	1003	0074
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																								X	X
Hepatocellular adenoma																									
Histiocytic sarcoma		X																							
Mesentery											+					+									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign					X									X					X	X	X		X	X	
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma														X											
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X				X						X							X		X	
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																X									
C-cell, carcinoma																									
Follicular cell, adenoma																									
Follicular cell, carcinoma																									
General Body System																									
Peritoneum																	+								
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma							X	X		X	X		X				X	X	X					X	X
Interstitial cell, adenoma						X						X		X		X				X	X	X	X		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 280 ppm

Number of Days on Study	704	714	716	725	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	081	070	077	073	065	080	086	093	098	106	107	108	113	118	120	068	082	083	084	089	090	096	105	109	110	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma									X																	3
Hepatocellular adenoma							X																			1
Histiocytic sarcoma																										1
Mesentery				+			+				+		+											+		7
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign	X			X					X				X													11
Bilateral, pheochromocytoma benign		X	X																							2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma					X							X				X									X	5
Carcinoma			X																							1
Parathyroid gland	M	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma						X							X				X						X			9
Pars intermedia, adenoma																X										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma					X					X				X	X											5
C-cell, carcinoma																							X			1
Follicular cell, adenoma					X																					1
Follicular cell, carcinoma				X							X															2
General Body System																										
Peritoneum																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																				X						1
Carcinoma			X																							2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X		X	X		X	X			X	30
Interstitial cell, adenoma								X									X			X			X	X		13

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 280 ppm

Number of Days on Study	462	464	491	506	511	515	557	561	584	596	610	610	617	630	631	632	636	638	665	673	679	679	679	690	704
Carcass ID Number	1011	1012	0701	0104	1017	1015	0572	0066	0967	0663	0662	0669	0779	0991	0992	0661	0619	0887	1001	1016	0664	0675	0678	1003	0704
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Lymph node		+	+	+	+	+	+	+					+	+	+	+	+	+	+	+	+			+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	I	M	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	I	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Basal cell carcinoma																									
Keratoacanthoma																		X			X				
Squamous cell papilloma																									
Sebaceous gland, adenoma																									
Subcutaneous tissue, fibroma									X						X						X				
Subcutaneous tissue, fibrosarcoma																							X		
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant		X																							
Oligodendroglioma malignant											X														
Pineal gland, meningioma malignant																									
Peripheral nerve											+			+				+				+			
Spinal cord											+			+				+				+			
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									
Histiocytic sarcoma		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye															+										
Zymbal's gland	+																								
Carcinoma	X																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 280 ppm

Number of Days on Study	704	714	716	725	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	081	070	077	073	065	080	086	093	098	106	107	108	113	118	120	068	082	083	084	089	090	096	105	109	110	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node	+								+															+		21
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibroadenoma						X				X																2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma														X												1
Basal cell carcinoma													X													1
Keratoacanthoma																X										3
Squamous cell papilloma																							X			1
Sebaceous gland, adenoma		X																								1
Subcutaneous tissue, fibroma																										3
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, lipoma					X																					1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Oligodendroglioma malignant																										1
Pineal gland, meningioma malignant																							X			1
Peripheral nerve				+																						5
Spinal cord				+																						5
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma									X																	1
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										1
Zymbal's gland																										1
Carcinoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 280 ppm

Number of Days on Study	462	464	491	506	511	515	557	561	584	596	610	610	617	630	631	632	636	638	665	673	679	679	679	690	704
Carcass ID Number	11 11	11 12	07 71	01 04	11 17	11 15	07 72	06 66	09 97	06 63	06 62	06 69	07 79	09 91	09 92	06 61	11 19	08 87	01 01	11 16	06 64	07 75	07 78	10 93	07 74
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, stromal nephroma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X																							
Leukemia mononuclear			X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
Mesothelioma malignant																	X								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 280 ppm

Number of Days on Study	704	714	716	725	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	081	070	077	073	065	080	086	093	098	106	107	108	113	118	120	068	082	083	084	089	090	096	105	109	110	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, stromal nephroma																				X						1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear	X	X		X					X	X				X		X				X	X			X		31
Mesothelioma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 830 ppm

Number of Days on Study	368	373	483	510	533	550	575	602	603	605	609	617	617	624	631	638	644	645	645	648	673	689	696	700	700
Carcass ID Number	172	177	161	128	163	168	156	146	124	173	178	134	136	153	155	140	154	132	169	149	152	139	174	127	137
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, spleen												X													
Hepatocellular carcinoma										X															
Hepatocellular adenoma																									
Histiocytic sarcoma															X										
Mesentery		+		+				+						+	+			+		+			+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, spleen												X													
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																	X								
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								X	
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign																X				X					
Bilateral, pheochromocytoma benign																					X				
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma											X														
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	M	+	+	+	+	+	+
Adenoma																									
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X								X		X											X	
Pars intermedia, adenoma																X									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma							X							X											
C-cell, carcinoma																									
Follicular cell, carcinoma																									
General Body System																									
Peritoneum									+								+					+			
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X																						
Carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 830 ppm

Number of Days on Study	704	704	720	725	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	144	145	166	138	130	131	143	148	157	158	170	171	179	180	122	125	126	133	135	147	151	160	164	165	175	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, spleen																										1
Hepatocellular carcinoma			X																					X		3
Hepatocellular adenoma											X															1
Histiocytic sarcoma																										1
Mesentery	+						+										+				+	+				13
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, spleen																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																										1
Stomach, forestomach	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant									X																	1
Pheochromocytoma benign	X							X		X																5
Bilateral, pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49
Adenoma																					X					1
Carcinoma																										1
Parathyroid gland	+	+	+	+	M	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Adenoma							X																			1
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma			X					X											X		X		X	X		10
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma			X																							3
C-cell, carcinoma							X																			1
Follicular cell, carcinoma				X									X													2
General Body System																										
Peritoneum																										3
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X																								2
Carcinoma								X																		1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma									X																	1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 830 ppm

Number of Days on Study	368	373	483	510	533	550	575	602	603	605	609	617	617	624	631	638	644	645	645	648	673	689	696	700	700
Carcass ID Number	172	177	161	128	163	168	156	446	224	173	178	134	136	153	155	140	154	132	169	149	152	139	174	127	137
Genital System (continued)																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma					X	X	X	X		X	X	X			X	X	X	X	X	X	X	X	X		X
Interstitial cell, adenoma				X					X				X	X										X	
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+				+	+	+				+	+	+	+	+		+	+	+	+	+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma												X													
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	I	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																				X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue, fibroma															X										X
Subcutaneous tissue, histiocytic sarcoma															X										
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma	X																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+					+			+				+		+							+		+	
Spinal cord	+					+			+				+		+							+		+	
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																+									
Eye																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Leukemia mononuclear		X		X		X		X	X	X				X	X	X		X	X	X	X	X	X		
Mesothelioma malignant									X								X					X			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 830 ppm

Number of Days on Study	704	704	720	725	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	144	145	166	138	130	131	143	148	157	158	170	171	179	180	122	125	126	133	135	147	151	160	164	165	175	Total Tissues/ Tumors
Genital System (continued)																										
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X			X	X	X	X	X	X	X		X	X	X	X	X	X	X	X		X		X	X	37
Interstitial cell, adenoma												X											X			7
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+				+																			18
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibroadenoma														X												2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Keratoacanthoma						X							X					X								3
Subcutaneous tissue, fibroma																										2
Subcutaneous tissue, histiocytic sarcoma																										1
Subcutaneous tissue, lipoma	X																									1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																								+		8
Spinal cord																								+		8
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Eye																								+		1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra																									+	1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear	X	X	X		X		X			X	X		X	X					X	X	X			X	X	29
Mesothelioma malignant																										3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 2,500 ppm

Number of Days on Study	266	449	492	539	590	597	602	605	607	617	617	634	645	654	668	700	702	711	711	720	729	729	729	729	729
Carcass ID Number	204	185	189	190	233	239	209	205	222	215	227	216	225	219	196	203	199	186	194	223	187	188	197	198	206
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, skin																			X						
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Sarcoma																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma												X													
Hepatocellular adenoma																									
Mesentery	+				+		+			+							+	+						+	
Sarcoma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Squamous cell papilloma												X													
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, skin																			X						
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign				X										X										X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								X	
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma													X		X						X				X
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
C-cell, carcinoma										X															
General Body System																									
Peritoneum											+														
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																						X			
Carcinoma										X	X											X			
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X		X	X			X	X	X	X		X		X	X		X	X	X		X	X	X
Interstitial cell, adenoma		X		X			X	X							X			X				X			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 2,500 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	207	211	213	229	230	235	236	237	181	183	184	191	193	195	200	201	202	208	228	220	221	232	234	238	240	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Schwannoma malignant, metastatic, skin																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma	X																									1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cholangiocarcinoma																										1
Hepatocellular adenoma				X																		X				2
Mesentery					+	+	+			+			+				+				+			+		15
Sarcoma					X												X									2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant, metastatic, skin																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																			X							1
Pheochromocytoma benign						X								X						X	X		X		X	9
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	M	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X			X		X			X	X		X		X							X			X		13
Pars intermedia, adenoma											X															1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma	X	X								X																3
C-cell, carcinoma																										1
General Body System																										
Peritoneum																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma						X							X													3
Carcinoma																										3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X																			X		X			3
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X		X	X	X	X	X		X	X	X				X		X	X	X	X	X	X	X	X	35
Interstitial cell, adenoma			X						X				X	X	X		X									13

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 2,500 ppm

Number of Days on Study	264	449	492	539	590	597	602	605	607	617	617	634	645	654	668	700	702	711	711	720	729	729	729	729	729	729
Carcass ID Number	204	185	189	190	233	239	209	205	222	215	227	216	225	219	196	203	199	186	194	223	187	188	197	198	206	
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Schwannoma malignant, metastatic, skin																			X							
Lymph node				+		+		+								+	+	+		+	+			+		
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Schwannoma malignant, metastatic, skin																			X							
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	
Fibroadenoma																X						X				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Basal cell carcinoma																										
Keratoacanthoma																										
Trichoepithelioma					X																					
Squamous cell carcinoma																										
Squamous cell papilloma																	X									
Subcutaneous tissue, fibroma								X					X													
Subcutaneous tissue, schwannoma malignant																			X							
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Osteosarcoma																										
Skeletal muscle												+							+							
Schwannoma malignant, metastatic, skin																			X							
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Peripheral nerve														+				+		+						
Spinal cord														+				+		+						
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Osteosarcoma, metastatic, bone																										
Schwannoma malignant, metastatic, skin																			X							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Squamous cell carcinoma			X																							
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Special Senses System																										
Zymbal's gland																										
Carcinoma																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Urethra																			+							
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Leukemia mononuclear	X			X		X	X	X			X			X	X	X	X	X		X	X	X				
Mesothelioma malignant											X															

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Emodin: 2,500 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	
Carcass ID Number	207	211	213	229	230	235	236	237	181	183	184	191	193	195	200	201	202	208	228	220	221	232	234	238	240	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant, metastatic, skin																										1
Lymph node					+	+																				11
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant, metastatic, skin																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibroadenoma																		X								3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	49
Basal cell carcinoma																								X		1
Keratoacanthoma	X		X																			X				3
Trichoepithelioma																										1
Squamous cell carcinoma														X												1
Squamous cell papilloma		X																								2
Subcutaneous tissue, fibroma										X	X			X												5
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma													X													1
Skeletal muscle																										2
Schwannoma malignant, metastatic, skin																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										3
Spinal cord																										3
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, bone													X													1
Schwannoma malignant, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma				X																						2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Zymbal's gland		+																								1
Carcinoma		X																								1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X												X				X								X	18
Mesothelioma malignant																										1

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	7/50 (14%)	13/49 (27%)	6/50 (12%)	9/50 (18%)
Adjusted rate[b]	17.0%	32.0%	14.9%	20.8%
Terminal rate[c]	6/30 (20%)	2/20 (10%)	2/21 (10%)	7/30 (23%)
First incidence (days)	686	511	638	539
Poly-3 test[d]	P=0.465N	P=0.091	P=0.513N	P=0.437
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	8/50 (16%)	13/49 (27%)	7/50 (14%)	10/50 (20%)
Adjusted rate	19.3%	32.0%	17.3%	23.1%
Terminal rate	6/30 (20%)	2/20 (10%)	3/21 (14%)	8/30 (27%)
First incidence (days)	646	511	638	539
Poly-3 test	P=0.514N	P=0.142	P=0.520N	P=0.438
Liver: Hepatocellular Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	7.6%	7.5%	0.0%
Terminal rate	0/30 (0%)	1/21 (5%)	1/21 (5%)	0/30 (0%)
First incidence (days)	—[e]	690	605	—
Poly-3 test	P=0.277N	P=0.113	P=0.114	—[f]
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	1/50 (2%)	4/50 (8%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.4%	10.1%	10.0%	4.7%
Terminal rate	1/30 (3%)	2/21 (10%)	2/21 (10%)	2/30 (7%)
First incidence (days)	729 (T)	690	605	729 (T)
Poly-3 test	P=0.517N	P=0.168	P=0.170	P=0.513
Mammary Gland: Fibroadenoma				
Overall rate	1/50 (2%)	2/50 (4%)	2/50 (4%)	3/50 (6%)
Adjusted rate	2.4%	5.1%	5.0%	7.1%
Terminal rate	1/30 (3%)	2/21 (10%)	1/21 (5%)	2/30 (7%)
First incidence (days)	729 (T)	729 (T)	648	700
Poly-3 test	P=0.291	P=0.487	P=0.490	P=0.319
Pancreatic Islets: Adenoma				
Overall rate	2/50 (4%)	5/50 (10%)	1/49 (2%)	1/50 (2%)
Adjusted rate	4.9%	12.6%	2.6%	2.4%
Terminal rate	2/30 (7%)	4/21 (19%)	1/20 (5%)	1/30 (3%)
First incidence (days)	729 (T)	630	729 (T)	729 (T)
Poly-3 test	P=0.170N	P=0.203	P=0.522N	P=0.487N
Pancreatic Islets: Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/49 (2%)	0/50 (0%)
Adjusted rate	7.3%	2.5%	2.6%	0.0%
Terminal rate	3/30 (10%)	0/21 (0%)	0/20 (0%)	0/30 (0%)
First incidence (days)	729 (T)	716	609	—
Poly-3 test	P=0.105N	P=0.317N	P=0.323N	P=0.112N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	6/50 (12%)	2/49 (4%)	1/50 (2%)
Adjusted rate	12.2%	15.1%	5.1%	2.4%
Terminal rate	5/30 (17%)	4/21 (19%)	1/20 (5%)	1/30 (3%)
First incidence (days)	729 (T)	630	609	729 (T)
Poly-3 test	P=0.036N	P=0.482	P=0.235N	P=0.092N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	15/49 (31%)	9/50 (18%)	10/48 (21%)	13/50 (26%)
Adjusted rate	36.5%	21.8%	25.2%	30.3%
Terminal rate	10/29 (35%)	4/21 (19%)	5/20 (25%)	11/30 (37%)
First incidence (days)	604	511	483	645
Poly-3 test	P=0.556	P=0.107N	P=0.193N	P=0.353N
Preputial Gland: Adenoma				
Overall rate	5/50 (10%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	11.8%	2.5%	5.0%	7.1%
Terminal rate	2/30 (7%)	1/21 (5%)	0/21 (0%)	3/30 (10%)
First incidence (days)	472	729 (T)	483	729 (T)
Poly-3 test	P=0.517N	P=0.117N	P=0.235N	P=0.353N
Preputial Gland: Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	7.3%	5.1%	2.5%	6.9%
Terminal rate	2/30 (7%)	0/21 (0%)	1/21 (5%)	1/30 (3%)
First incidence (days)	590	704	729 (T)	617
Poly-3 test	P=0.541	P=0.520N	P=0.322N	P=0.642N
Preputial Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	3/50 (6%)	3/50 (6%)	5/50 (10%)
Adjusted rate	18.7%	7.6%	7.5%	11.6%
Terminal rate	4/30 (13%)	1/21 (5%)	1/21 (5%)	3/30 (10%)
First incidence (days)	472	704	483	617
Poly-3 test	P=0.411N	P=0.121N	P=0.115N	P=0.266N
Prostate Gland: Adenoma				
Overall rate	1/50 (2%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.4%	0.0%	2.5%	7.1%
Terminal rate	1/30 (3%)	0/21 (0%)	1/21 (5%)	3/30 (10%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P=0.090	P=0.507N	P=0.753	P=0.318
Skin: Keratoacanthoma				
Overall rate	3/50 (6%)	3/50 (6%)	3/50 (6%)	3/50 (6%)
Adjusted rate	7.3%	7.5%	7.6%	7.1%
Terminal rate	2/30 (7%)	1/21 (5%)	3/21 (14%)	3/30 (10%)
First incidence (days)	704	638	729 (T)	729 (T)
Poly-3 test	P=0.575N	P=0.652	P=0.645	P=0.647N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	3/50 (6%)	4/50 (8%)	3/50 (6%)	5/50 (10%)
Adjusted rate	7.3%	10.0%	7.6%	11.8%
Terminal rate	2/30 (7%)	2/21 (10%)	3/21 (14%)	4/30 (13%)
First incidence (days)	704	638	729 (T)	702
Poly-3 test	P=0.343	P=0.486	P=0.645	P=0.376
Skin: Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	1/50 (2%)	2/50 (4%)	0/50 (0%)	2/50 (4%)
Adjusted rate	2.5%	5.1%	0.0%	4.7%
Terminal rate	1/30 (3%)	2/21 (10%)	0/21 (0%)	1/30 (3%)
First incidence (days)	729 (T)	729 (T)	—	590
Poly-3 test	P=0.480	P=0.487	P=0.507N	P=0.517

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	3/50 (6%)	4/50 (8%)	3/50 (6%)	6/50 (12%)
Adjusted rate	7.3%	10.0%	7.6%	14.1%
Terminal rate	2/30 (7%)	2/21 (10%)	3/21 (14%)	5/30 (17%)
First incidence (days)	704	638	729 (T)	702
Poly-3 test	P=0.343	P=0.486	P=0.645	P=0.259
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	4/50 (8%)	6/50 (12%)	3/50 (6%)	8/50 (16%)
Adjusted rate	9.8%	15.0%	7.6%	18.6%
Terminal rate	3/30 (10%)	4/21 (19%)	3/21 (14%)	6/30 (20%)
First incidence (days)	704	638	729 (T)	590
Poly-3 test	P=0.172	P=0.352	P=0.521N	P=0.198
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	5/50 (10%)
Adjusted rate	2.4%	7.4%	5.0%	11.6%
Terminal rate	0/30 (0%)	0/21 (0%)	0/21 (0%)	3/30 (10%)
First incidence (days)	704	584	631	605
Poly-3 test	P=0.100	P=0.300	P=0.491	P=0.113
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma				
Overall rate	2/50 (4%)	4/50 (8%)	2/50 (4%)	5/50 (10%)
Adjusted rate	4.8%	9.8%	5.0%	11.6%
Terminal rate	0/30 (0%)	0/21 (0%)	0/21 (0%)	3/30 (10%)
First incidence (days)	122	584	631	605
Poly-3 test	P=0.227	P=0.322	P=0.676	P=0.226
Testes: Adenoma				
Overall rate	41/50 (82%)	43/50 (86%)	44/50 (88%)	48/50 (96%)
Adjusted rate	92.5%	94.2%	92.6%	98.5%
Terminal rate	29/30 (97%)	21/21 (100%)	20/21 (95%)	30/30 (100%)
First incidence (days)	528	555	510	449
Poly-3 test	P=0.099	P=0.553	P=0.662	P=0.135
Thyroid Gland (C-Cell): Adenoma				
Overall rate	3/50 (6%)	5/50 (10%)	3/50 (6%)	3/50 (6%)
Adjusted rate	7.3%	12.6%	7.4%	7.1%
Terminal rate	3/30 (10%)	4/21 (19%)	0/21 (0%)	3/30 (10%)
First incidence (days)	729 (T)	632	575	729 (T)
Poly-3 test	P=0.426N	P=0.340	P=0.657	P=0.646N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	4/50 (8%)	6/50 (12%)	4/50 (8%)	4/50 (8%)
Adjusted rate	9.8%	15.1%	9.9%	9.3%
Terminal rate	3/30 (10%)	5/21 (24%)	1/21 (5%)	3/30 (10%)
First incidence (days)	711	632	575	617
Poly-3 test	P=0.420N	P=0.350	P=0.636	P=0.619N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	2.4%	7.6%	5.1%	0.0%
Terminal rate	1/30 (3%)	2/21 (10%)	1/21 (5%)	0/30 (0%)
First incidence (days)	729 (T)	725	725	—
Poly-3 test	P=0.174N	P=0.292	P=0.488	P=0.493N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
All Organs: Mononuclear Cell Leukemia				
Overall rate	28/50 (56%)	31/50 (62%)	29/50 (58%)	18/50 (36%)
Adjusted rate	61.5%	65.1%	63.7%	38.7%
Terminal rate	16/30 (53%)	7/21 (33%)	11/21 (52%)	6/30 (20%)
First incidence (days)	284	491	373	266
Poly-3 test	P=0.003N	P=0.442	P=0.500	P=0.021N
All Organs: Malignant Mesothelioma				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.8%	2.5%	7.4%	2.3%
Terminal rate	0/30 (0%)	0/21 (0%)	0/21 (0%)	0/30 (0%)
First incidence (days)	604	636	603	617
Poly-3 test	P=0.440N	P=0.515N	P=0.487	P=0.489N
All Organs: Benign Neoplasms				
Overall rate	45/50 (90%)	46/50 (92%)	47/50 (94%)	49/50 (98%)
Adjusted rate	98.3%	97.6%	97.4%	99.9%
Terminal rate	30/30 (100%)	21/21 (100%)	21/21 (100%)	30/30 (100%)
First incidence (days)	472	511	483	449
Poly-3 test	P=0.292	P=0.792N	P=0.698N	P=0.656
All Organs: Malignant Neoplasms				
Overall rate	36/50 (72%)	38/50 (76%)	36/50 (72%)	29/50 (58%)
Adjusted rate	74.8%	76.7%	75.7%	59.8%
Terminal rate	20/30 (67%)	10/21 (48%)	13/21 (62%)	12/30 (40%)
First incidence (days)	122	462	368	266
Poly-3 test	P=0.027N	P=0.504	P=0.553	P=0.084N
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	99.9%	100.0%	100.0%	100.0%
Terminal rate	30/30 (100%)	21/21 (100%)	21/21 (100%)	30/30 (100%)
First incidence (days)	122	462	368	266
Poly-3 test	P=1.000	P=1.000	P=1.000	P=1.000

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, pancreatic islets, pituitary gland, preputial gland, prostate gland, testis, 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 are the P values 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**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE A4a
Historical Incidence of Nasal Squamous Cell Carcinoma in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Southern Research Institute	
2,2-Bis(bromomethyl)-1,3-propanediol	0/51
Benzyl acetate	0/50
Butyl benzyl phthalate	0/50
D&C Yellow No. 11	0/50
o-Nitroanisole	1/50
p-Nitrobenzoic acid	1/50
Overall Historical Incidence	
Total (%)	4/904 (0.4%)
Mean ± standard deviation	0.4% ± 0.9%
Range	0%-2%

[a] Data as of 10 November 1998

TABLE A4b
Historical Incidence of Mononuclear Cell Leukemia in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Southern Research Institute	
2,2-Bis(bromomethyl)-1,3-propanediol	27/51
Benzyl acetate	16/50
Butyl benzyl phthalate	31/50
D&C Yellow No. 11	37/50
o-Nitroanisole	26/50
p-Nitrobenzoic acid	29/50
Overall Historical Incidence	
Total (%)	494/904 (54.7%)
Mean ± standard deviation	54.7% ± 11.2%
Range	32%-74%

[a] Data as of 10 November 1998; includes data for lymphocytic, monocytic, and undifferentiated leukemia

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

	0 ppm	280 ppm	830 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	65	65	65	65
6-Month interim evaluation	10	10	10	10
12-Month interim evaluation	5	5	5	5
Early deaths				
Moribund	17	26	27	16
Natural deaths	3	3	2	4
Survivors				
Terminal sacrifice	30	21	21	30
Animals examined microscopically	65	65	65	65
6-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Basophilic focus			1 (10%)	
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Nephropathy	9 (90%)	9 (90%)	10 (100%)	8 (80%)
Renal tubule, hyaline droplet		10 (100%)	10 (100%)	10 (100%)
Renal tubule, pigmentation	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Systems Examined with No Lesions Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
12-Month Interim Evaluation				
Alimentary System				
Liver	(5)	(5)	(5)	(5)
Basophilic focus	1 (20%)		1 (20%)	1 (20%)
Infiltration cellular, mixed cell	2 (40%)		1 (20%)	1 (20%)
Mixed cell focus				1 (20%)
Bile duct, hyperplasia	1 (20%)			

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

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
12-Month Interim Evaluation (continued)				
Urinary System				
Kidney	(5)	(5)	(5)	(5)
Inflammation, chronic	1 (20%)			1 (20%)
Nephropathy	5 (100%)	5 (100%)	5 (100%)	5 (100%)
Renal tubule, hyaline droplet		5 (100%)	5 (100%)	5 (100%)
Renal tubule, pigmentation	5 (100%)	5 (100%)	5 (100%)	4 (80%)
Systems Examined with No Lesions Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
2-Year Study				
Alimentary System				
Intestine large, colon	(49)	(50)	(50)	(50)
Congestion		1 (2%)		
Edema		1 (2%)		
Inflammation, chronic				1 (2%)
Epithelium, hyperplasia				1 (2%)
Intestine large, cecum	(49)	(50)	(50)	(49)
Congestion		1 (2%)		
Edema	2 (4%)	2 (4%)	3 (6%)	2 (4%)
Inflammation, chronic				2 (4%)
Epithelium, hyperplasia				2 (4%)
Intestine small, jejunum	(49)	(48)	(50)	(49)
Congestion		1 (2%)		
Epithelium, hyperplasia				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	5 (10%)	5 (10%)		11 (22%)
Basophilic focus	20 (40%)	18 (36%)	18 (36%)	23 (46%)
Clear cell focus	7 (14%)	7 (14%)	9 (18%)	13 (26%)
Degeneration, cystic	7 (14%)	10 (20%)	11 (22%)	10 (20%)
Eosinophilic focus	6 (12%)	8 (16%)	6 (12%)	10 (20%)
Hematopoietic cell proliferation	2 (4%)			
Hemorrhage			1 (2%)	
Hepatodiaphragmatic nodule	3 (6%)	5 (10%)	2 (4%)	4 (8%)
Infiltration cellular, mixed cell		2 (4%)	2 (4%)	2 (4%)
Mixed cell focus	6 (12%)	4 (8%)	3 (6%)	9 (18%)
Necrosis, focal	2 (4%)	2 (4%)	2 (4%)	
Bile duct, hyperplasia	33 (66%)	24 (48%)	20 (40%)	14 (28%)
Centrilobular, necrosis	1 (2%)	1 (2%)		
Hepatocyte, vacuolization cytoplasmic	3 (6%)	3 (6%)	4 (8%)	2 (4%)
Kupffer cell, pigmentation	1 (2%)	1 (2%)	2 (4%)	4 (8%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Mesentery	(8)	(7)	(13)	(15)
Accessory spleen	4 (50%)	2 (29%)	2 (15%)	
Fat, necrosis	5 (63%)	6 (86%)	11 (85%)	12 (80%)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	23 (46%)	19 (38%)	16 (32%)	23 (46%)
Metaplasia, hepatocyte	1 (2%)		1 (2%)	
Acinus, hyperplasia, focal	1 (2%)	1 (2%)		1 (2%)
Salivary glands	(50)	(50)	(50)	(49)
Edema		1 (2%)		
Stomach, forestomach	(50)	(50)	(49)	(48)
Diverticulum	1 (2%)			
Edema	4 (8%)	3 (6%)	2 (4%)	5 (10%)
Perforation			1 (2%)	
Ulcer	6 (12%)	2 (4%)	4 (8%)	4 (8%)
Epithelium, hyperplasia	5 (10%)	4 (8%)	7 (14%)	5 (10%)
Stomach, glandular	(50)	(50)	(49)	(48)
Edema	1 (2%)	3 (6%)		
Erosion	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Ulcer	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Epithelium, hyperplasia		1 (2%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	34 (68%)	31 (62%)	35 (70%)	36 (72%)
Thrombosis		2 (4%)	4 (8%)	2 (4%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	13 (26%)	18 (36%)	16 (32%)	10 (20%)
Degeneration, fatty	7 (14%)	9 (18%)	10 (20%)	8 (16%)
Hyperplasia, diffuse		2 (4%)	1 (2%)	1 (2%)
Hyperplasia, focal	3 (6%)		4 (8%)	2 (4%)
Hypertrophy			1 (2%)	
Hypertrophy, focal	6 (12%)	9 (18%)	7 (14%)	3 (6%)
Necrosis		1 (2%)		
Adrenal medulla	(50)	(49)	(50)	(50)
Hyperplasia	10 (20%)	9 (18%)	7 (14%)	17 (34%)
Islets, pancreatic	(50)	(50)	(49)	(50)
Hyperplasia	5 (10%)		1 (2%)	2 (4%)
Parathyroid gland	(48)	(45)	(45)	(47)
Hyperplasia, focal		1 (2%)		
Pituitary gland	(49)	(50)	(48)	(50)
Pars distalis, angiectasis	1 (2%)		1 (2%)	1 (2%)
Pars distalis, cyst	7 (14%)	9 (18%)	5 (10%)	3 (6%)
Pars distalis, hyperplasia, focal	6 (12%)	3 (6%)	5 (10%)	1 (2%)
Pars intermedia, cyst		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Ultimobranchial cyst			1 (2%)	1 (2%)
C-Cell, hyperplasia	7 (14%)	2 (4%)	7 14%)	4 (8%)
Follicle, cyst	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Follicular cell, hyperplasia	1 (2%)			

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Atypia cellular	29 (58%)	26 (52%)	25 (50%)	29 (58%)
Preputial gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)		1 (2%)	
Inflammation, chronic	17 (34%)	20 (40%)	13 (26%)	20 (40%)
Prostate	(50)	(50)	(50)	(50)
Fibrosis	5 (10%)	5 (10%)	4 (8%)	6 (12%)
Inflammation, chronic	27 (54%)	24 (48%)	28 (56%)	29 (58%)
Epithelium, hyperplasia	12 (24%)	3 (6%)	10 (20%)	9 (18%)
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, atrophy	7 (14%)	12 (24%)	3 (6%)	8 (16%)
Interstitial cell, hyperplasia	4 (8%)	8 (16%)	3 (6%)	5 (10%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	5 (10%)	3 (6%)	11 (22%)
Lymph node	(12)	(21)	(18)	(11)
Iliac, pigmentation		1 (5%)		1 (9%)
Mediastinal, hemorrhage	1 (8%)	1 (5%)	2 (11%)	4 (36%)
Mediastinal, hyperplasia, lymphoid	1 (8%)		1 (6%)	
Mediastinal, pigmentation		2 (10%)	4 (22%)	2 (18%)
Pancreatic, hemorrhage				1 (9%)
Pancreatic, pigmentation		1 (5%)		
Renal, hemorrhage			1 (6%)	
Renal, pigmentation			1 (6%)	1 (9%)
Lymph node, mandibular	(49)	(50)	(48)	(50)
Ectasia		3 (6%)	2 (4%)	1 (2%)
Hemorrhage			1 (2%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)	
Pigmentation		2 (4%)		2 (4%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Ectasia				6 (12%)
Hemorrhage	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Hyperplasia, lymphoid				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Accessory spleen	1 (2%)		1 (2%)	
Fibrosis	8 (16%)	9 (18%)	3 (6%)	3 (6%)
Hematopoietic cell proliferation	3 (6%)	5 (10%)	4 (8%)	8 (16%)
Necrosis	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Pigmentation	1 (2%)	2 (4%)	4 (8%)	4 (8%)
Integumentary System				
Mammary gland	(49)	(47)	(49)	(49)
Hyperplasia	14 (29%)	23 (49%)	10 (20%)	13 (27%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Integumentary System (continued)				
Skin	(50)	(50)	(49)	(49)
Cyst epithelial inclusion			3 (6%)	2 (4%)
Hyperkeratosis	2 (4%)	3 (6%)	1 (2%)	
Hyperplasia, basal cell		1 (2%)		
Inflammation, chronic	2 (4%)	2 (4%)		1 (2%)
Ulcer		2 (4%)		
Epidermis, hyperplasia	1 (2%)	3 (6%)		
Subcutaneous tissue, fibrosis		1 (2%)		
Subcutaneous tissue, inflammation, chronic active				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteopetrosis	1 (2%)			
Skeletal muscle	(1)			(2)
Hemorrhage				1 (50%)
Necrosis				1 (50%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Compression	4 (8%)	2 (4%)	5 (10%)	5 (10%)
Hydrocephalus	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Necrosis	1 (2%)	1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)	2 (4%)	1 (2%)
Infiltration cellular, histiocyte	26 (52%)	28 (56%)	27 (54%)	35 (70%)
Inflammation, chronic	1 (2%)			2 (4%)
Alveolar epithelium, hyperplasia	5 (10%)	1 (2%)	2 (4%)	5 (10%)
Nose	(50)	(50)	(50)	(50)
Foreign body	4 (8%)	3 (6%)	1 (2%)	2 (4%)
Inflammation, chronic	9 (18%)	8 (16%)	9 (18%)	8 (16%)
Respiratory epithelium, hyperplasia	6 (12%)	7 (14%)	8 (16%)	5 (10%)
Respiratory epithelium, metaplasia, squamous	4 (8%)	3 (6%)	5 (10%)	1 (2%)
Special Senses System				
Eye	(3)	(1)	(1)	
Atrophy	1 (33%)			
Cataract	2 (67%)	1 (100%)	1 (100%)	
Retina, degeneration	2 (67%)	1 (100%)	1 (100%)	

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst		1 (2%)	2 (4%)	1 (2%)
Hydronephrosis				2 (4%)
Infarct				3 (6%)
Inflammation, chronic	15 (30%)	11 (22%)	13 (26%)	25 (50%)
Nephropathy	48 (96%)	50 (100%)	50 (100%)	50 (100%)
Renal tubule, hyaline droplet	3 (6%)	45 (90%)	43 (86%)	43 (86%)
Renal tubule, hyperplasia			1 (2%)	
Renal tubule, necrosis		1 (2%)		1 (2%)
Renal tubule, pigmentation	35 (70%)	47 (94%)	49 (98%)	50 (100%)
Transitional epithelium, hyperplasia	4 (8%)	4 (8%)	3 (6%)	2 (4%)
Urinary bladder	(50)	(50)	(50)	(50)
Edema				1 (2%)
Hemorrhage				1 (2%)
Transitional epithelium, hyperplasia			1 (2%)	

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF EMODIN

TABLE B1 Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Emodin 104

TABLE B2 Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin 108

TABLE B3 Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Emodin 124

TABLE B4a Historical Incidence of Zymbal's Gland Carcinoma in Untreated Female F344/N Rats 127

TABLE B4b Historical Incidence of Mononuclear Cell Leukemia in Untreated Female F344/N Rats 127

TABLE B5 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Emodin 128

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

	0 ppm	280 ppm	830 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	65	65	65	65
6-Month interim evaluation	10	10	10	10
12-Month interim evaluation	5	5	5	5
Early deaths				
Moribund	12	8	11	15
Natural deaths	5	3	4	1
Survivors				
Terminal sacrifice	33	39	35	34
Animals examined microscopically	65	65	65	65
Systems Examined at 6 and 12 Months with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Intestine small, ileum	(48)	(50)	(49)	(50)
Liver	(49)	(50)	(50)	(50)
Hemangioma		1 (2%)		
Hepatocellular adenoma, multiple				1 (2%)
Pancreas	(49)	(50)	(49)	(50)
Hemangiosarcoma, metastatic, spleen			1 (2%)	
Stomach, forestomach	(49)	(50)	(50)	(50)
Stomach, glandular	(49)	(50)	(50)	(50)
Cardiovascular System				
Heart	(49)	(50)	(50)	(50)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Adenoma		1 (2%)		
Carcinoma				1 (2%)
Adrenal medulla	(47)	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)			
Pheochromocytoma complex				1 (2%)
Pheochromocytoma benign	1 (2%)		4 (8%)	1 (2%)
Islets, pancreatic	(49)	(50)	(49)	(50)
Adenoma	1 (2%)	1 (2%)	1 (2%)	
Carcinoma		1 (2%)	1 (2%)	
Pituitary gland	(49)	(50)	(49)	(49)
Pars distalis, adenoma	15 (31%)	21 (42%)	25 (51%)	25 (51%)
Pars intermedia, adenoma	1 (2%)			1 (2%)
Pars intermedia, carcinoma		1 (2%)		
Thyroid gland	(49)	(50)	(50)	(50)
Bilateral, C-cell, adenoma		1 (2%)		
C-cell, adenoma	4 (8%)	5 (10%)	4 (8%)	3 (6%)
C-cell, carcinoma	2 (4%)	2 (4%)		3 (6%)
Follicular cell, adenoma		1 (2%)		1 (2%)
Follicular cell, carcinoma		1 (2%)	1 (2%)	
General Body System				
Peritoneum				(1)
Genital System				
Clitoral gland	(49)	(50)	(49)	(49)
Adenoma	10 (20%)	10 (20%)	8 (16%)	9 (18%)
Carcinoma	2 (4%)	3 (6%)	2 (4%)	
Bilateral, adenoma		2 (4%)		1 (2%)
Ovary	(49)	(50)	(50)	(50)
Bilateral, granulosa cell tumor benign		1 (2%)		
Uterus	(49)	(50)	(50)	(50)
Leiomyoma			1 (2%)	
Polyp stromal	10 (20%)	7 (14%)	7 (14%)	10 (20%)
Polyp stromal, multiple		1 (2%)		
Sarcoma stromal	1 (2%)			
Vagina	(2)	(3)	(3)	(5)
Granular cell tumor benign			1 (33%)	
Squamous cell papilloma			1 (33%)	
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Lymph node	(5)	(3)	(4)	(3)
Lymph node, mandibular	(49)	(50)	(50)	(50)
Lymph node, mesenteric	(49)	(50)	(49)	(50)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Osteosarcoma, metastatic, bone	1 (2%)			
Thymus	(47)	(49)	(49)	(50)
Thymoma benign	1 (2%)		1 (2%)	
Thymoma malignant		1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Integumentary System				
Mammary gland	(49)	(50)	(50)	(50)
Carcinoma				2 (4%)
Fibroadenoma	18 (37%)	13 (26%)	20 (40%)	16 (32%)
Fibroadenoma, multiple	5 (10%)	5 (10%)		1 (2%)
Skin	(49)	(50)	(50)	(50)
Basal cell adenoma				1 (2%)
Keratoacanthoma	1 (2%)			
Schwannoma malignant	1 (2%)			
Subcutaneous tissue, fibroma	2 (4%)		1 (2%)	
Subcutaneous tissue, fibrosarcoma		1 (2%)		
Subcutaneous tissue, lipoma			1 (2%)	
Subcutaneous tissue, sarcoma			1 (2%)	
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Osteosarcoma	1 (2%)	1 (2%)		1 (2%)
Nervous System				
Brain	(49)	(50)	(50)	(50)
Glioma malignant			1 (2%)	
Granular cell tumor malignant		1 (2%)		
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)			
Alveolar/bronchiolar carcinoma	1 (2%)			
Carcinoma, metastatic, adrenal cortex				1 (2%)
Osteosarcoma, metastatic, bone	1 (2%)	1 (2%)		1 (2%)
Special Senses System				
Lacrimal gland	(1)			
Carcinoma	1 (100%)			
Zymbal's gland				(3)
Carcinoma				3 (100%)
Urinary System				
Kidney	(49)	(50)	(49)	(50)
Osteosarcoma, metastatic, bone				1 (2%)
Urinary bladder	(49)	(50)	(50)	(50)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	14 (28%)	17 (34%)	16 (32%)	3 (6%)
Mesothelioma malignant				1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]				
2-Year study	45	47	48	43
Total primary neoplasms				
2-Year study	94	99	98	85
Total animals with benign neoplasms				
2-Year study	41	39	41	37
Total benign neoplasms				
2-Year study	70	70	75	70
Total animals with malignant neoplasms				
2-Year study	22	24	22	15
Total malignant neoplasms				
2-Year study	24	29	23	15
Total animals with metastatic neoplasms				
2-Year study	1	1	1	2
Total metastatic neoplasms				
2-Year study	2	1	1	3

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	448	497	527	544	596	610	630	630	645	646	648	685	686	704	704	725	725	730	730	730	731	731	731	731	731
Carcass ID Number	291	251	265	258	277	252	260	272	281	274	275	279	298	280	297	243	289	253	254	256	246	247	248	249	250
Alimentary System																									
Esophagus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	M	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery															+										
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	A	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant			X																						
Pheochromocytoma benign															X										
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																					X				
Parathyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+
Pituitary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X			X		X			X	X	X				X		X							
Pars intermedia, adenoma																								X	
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma			X																	X			X		
C-cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma		X		X																	X				
Carcinoma														X											X
Ovary	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal								X	X		X	X	X										X		
Sarcoma stromal																X									
Vagina									+	+															

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	266	268	269	270	282	283	284	285	296	299	300	241	244	245	261	262	263	264	276	286	288	290	292	294	295	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Mesentery																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pheochromocytoma malignant																										1
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma			X		X					X		X								X		X	X			15
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma	X																									4
C-cell, carcinoma																				X			X			2
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma	X		X							X	X	X				X					X					10
Carcinoma																										2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp stromal			X	X			X							X												10
Sarcoma stromal																										1
Vagina																										2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	448	497	527	544	596	610	630	630	645	646	648	685	686	704	704	725	725	730	730	730	731	731	731	731	731
Carcass ID Number	291	251	265	258	277	252	260	272	281	274	275	279	298	280	297	243	289	253	254	256	246	247	248	249	250
Hematopoietic System																									
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+									+		+										
Lymph node, mandibular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma, metastatic, bone																									
Thymus	A	+	+	+	+	+	+	+	+	+	M	+	+	I	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign						X																			
Integumentary System																									
Mammary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma						X	X	X	X		X					X		X				X	X		X
Fibroadenoma, multiple					X					X					X										
Skin	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Schwannoma malignant																								X	
Subcutaneous tissue, fibroma																	X								
Musculoskeletal System																									
Bone	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																									
Nervous System																									
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve				+									+												
Spinal cord				+									+												
Respiratory System																									
Lung	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																							X		
Alveolar/bronchiolar carcinoma																							X		
Osteosarcoma, metastatic, bone																									
Nose	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye					+					+												+			
Lacrimal gland						+																			
Carcinoma						X																			
Urinary System																									
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X			X				X	X				X		X	X								X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	266	268	269	270	282	283	284	285	296	299	300	241	244	245	261	262	263	264	276	286	288	290	292	294	295	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node										+			+													5
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, bone															X											1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Thymoma benign																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibroadenoma		X							X			X	X		X	X							X		X	18
Fibroadenoma, multiple																					X	X				5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Keratoacanthoma							X																			1
Schwannoma malignant																										1
Subcutaneous tissue, fibroma													X													2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Osteosarcoma															X											1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Peripheral nerve																										2
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma																										1
Alveolar/bronchiolar carcinoma																										1
Osteosarcoma, metastatic, bone															X											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye									+								+									5
Lacrimal gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X			X							X			X								X		14

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 280 ppm

Number of Days on Study	396	572	575	600	605	624	634	646	656	690	716	730	730	730	731	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	311	316	309	303	354	305	334	304	332	337	340	301	302	348	312	313	314	315	326	327	328	329	330	336	338
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Mesentery							+															+			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X							
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X		X	X	X	X			X		X	X		X	X		X	X			X	
Pars intermedia, carcinoma		X																							
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma							X							X							X				
C-cell, carcinoma																									
Follicular cell, adenoma														X											
Follicular cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X		X					X						X		X	
Carcinoma							X																		
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, granulosa cell tumor benign																X									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal													X	X				X				X	X		
Polyp stromal, multiple												X													
Vagina			+			M		+																	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 280 ppm

Number of Days on Study	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	339	341	342	344	345	306	307	310	317	320	321	323	324	325	331	335	351	352	353	355	356	357	358	359	360	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma															X											1
Mesentery	+				+							+							+							6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																								+		1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma															X											1
Carcinoma							X																			1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X					X			X		X					X					X			X	X	21
Pars intermedia, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, C-cell, adenoma																		X								1
C-cell, adenoma	X																								X	5
C-cell, carcinoma								X											X							2
Follicular cell, adenoma																										1
Follicular cell, carcinoma										X																1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X									X				X					X				X	10
Carcinoma				X														X								3
Bilateral, adenoma					X														X							2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, granulosa cell tumor benign																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal	X													X												7
Polyp stromal, multiple																										1
Vagina																+										3

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 280 ppm

Number of Days on Study	396	572	575	600	605	624	634	646	656	690	716	730	730	730	731	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	311	316	309	303	354	305	334	304	332	337	340	301	302	348	312	313	314	315	326	327	328	329	330	336	338
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+			+	+																		
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma malignant				X																					
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma							X		X	X				X											
Fibroadenoma, multiple																					X			X	X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma	X																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor malignant											X														
Peripheral nerve											+														
Spinal cord											+														
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma, metastatic, bone	X																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye													+								+				
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X		X	X	X	X							X	X								X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 280 ppm

Number of Days on Study	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	339	341	342	344	345	306	307	310	317	320	321	323	324	325	331	335	351	352	353	355	356	357	358	359	360	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymoma malignant																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma			X		X		X	X	X					X				X				X			X	13
Fibroadenoma, multiple				X																				X		5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma								X																		1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granular cell tumor malignant																										1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X				X						X	X			X	X	X	X				X		17

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 830 ppm

Number of Days on Study	232	527	539	592	617	648	665	665	674	674	677	702	711	725	725	730	730	730	731	731	731	731	731	731	731
Carcass ID Number	369	411	381	391	378	386	362	399	409	420	365	402	397	383	408	393	394	395	371	372	373	374	375	382	384
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery														+											
Pancreas	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, spleen																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign							X													X				X	X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X												
Carcinoma															X										
Parathyroid gland	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma						X	X	X	X					X			X	X	X	X					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma				X																					
Follicular cell, carcinoma																					X				
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X									X								X	
Carcinoma							X																		
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma													X												
Polyp stromal		X								X			X										X		
Vagina					+		+																		
Granular cell tumor benign					X																				
Squamous cell papilloma																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 830 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	385	396	398	400	406	410	412	413	414	415	417	419	361	363	364	366	367	368	376	377	379	387	403	404	405	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma, metastatic, spleen			X																							1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma			X	X		X	X		X	X	X	X			X	X		X		X	X	X	X		X	25
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma							X								X				X							4
Follicular cell, carcinoma																										1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	49
Adenoma					X						X									X		X	X			8
Carcinoma														X												2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma																										1
Polyp stromal														X			X					X				7
Vagina				+																						3
Granular cell tumor benign																										1
Squamous cell papilloma				X																						1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 830 ppm

Number of Days on Study	232	527	539	592	617	648	665	665	674	674	677	702	711	725	725	730	730	730	731	731	731	731	731	731	731
Carcass ID Number	369	411	381	391	378	386	362	399	409	420	365	402	397	383	408	393	394	395	371	372	373	374	375	382	384
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+		+							+															
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma				X	X	X	X							X		X	X		X						
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma													X												
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, sarcoma	X																								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle															+										
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant																									
Peripheral nerve						+									+										
Spinal cord						+									+										
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye														+			+			+					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	X	X					X	X	X	X					X		X						

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 830 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	385	396	398	400	406	410	412	413	414	415	417	419	361	363	364	366	367	368	376	377	379	387	403	404	405	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node			+																							4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma			X																							1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymoma benign	X																									1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma	X						X	X			X	X	X	X	X			X	X	X					X	20
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, lipoma																					X					1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma malignant						X																				1
Peripheral nerve							+																			3
Spinal cord							+																			3
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye							+																			4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X				X		X		X			X		X					X						16

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 2,500 ppm

Number of Days on Study	360	529	590	592	597	602	603	610	618	646	657	665	674	689	690	716	730	730	730	730	730	731	731	731	731
Carcass ID Number	447	422	449	441	463	480	468	423	470	469	466	428	459	451	438	467	442	443	444	446	448	426	427	429	430
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma, multiple																									
Mesentery									+												+		+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma				X																					
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma complex																									
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X	X		X		X	X	X			X	X	X	X	X	X		X			X		
Pars intermedia, adenoma		X																							
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																		X							
C-cell, carcinoma										X						X								X	
Follicular cell, adenoma																			X						
General Body System																									
Peritoneum															+										
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma		X	X														X					X			
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal				X														X		X	X				
Vagina							+		+			+				+									+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 2,500 ppm

Number of Days on Study	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	456	457	458	460	479	421	431	433	434	435	436	437	439	440	452	453	454	455	461	462	464	465	473	474	475	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma, multiple																					X					1
Mesentery	+							+					+													6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma complex															X											1
Pheochromocytoma benign																X										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma				X		X	X		X			X		X			X	X			X	X		X		25
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma									X															X		3
C-cell, carcinoma																										3
Follicular cell, adenoma																										1
General Body System																										
Peritoneum																										1
Genital System																										
Clitoral gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma			X									X			X			X			X					9
Bilateral, adenoma				X																						1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal	X									X		X					X					X		X		10
Vagina																										5

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 2,500 ppm

Number of Days on Study	360	529	590	592	597	602	603	610	618	646	657	665	674	689	690	716	730	730	730	730	730	731	731	731	731
Carcass ID Number	447	422	449	441	463	480	468	423	470	469	466	428	459	451	438	467	442	443	444	446	448	426	427	429	430
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+			+					+													
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																				X					
Fibroadenoma		X				X		X		X			X									X	X		X
Fibroadenoma, multiple																X									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma											X														
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, adrenal cortex				X																					
Osteosarcoma, metastatic, bone											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye							+	+																	
Zymbal's gland	+		+		+																				
Carcinoma	X		X		X																				
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma, metastatic, bone											X														
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X					X													
Mesothelioma malignant															X										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Emodin: 2,500 ppm

Number of Days on Study	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	456	457	458	460	479	421	431	433	434	435	436	437	439	440	452	453	454	455	461	462	464	465	473	474	475	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma									X																	2
Fibroadenoma				X	X		X		X	X										X	X	X				16
Fibroadenoma, multiple																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																					X					1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, adrenal cortex																										1
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																			+							1
Eye																										2
Zymbal's gland																M										3
Carcinoma																										3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, bone																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X																3
Mesothelioma malignant																										1

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	1/47 (2%)	0/50 (0%)	4/50 (8%)	1/50 (2%)
Adjusted rate[b]	2.3%	0.0%	8.8%	2.3%
Terminal rate[c]	0/33 (0%)	0/39 (0%)	3/35 (9%)	1/34 (3%)
First incidence (days)	704	—[e]	665	730 (T)
Poly-3 test[d]	P=0.564	P=0.487N	P=0.197	P=0.755N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	2/47 (4%)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted rate	4.6%	0.0%	8.8%	4.5%
Terminal rate	0/33 (0%)	0/39 (0%)	3/35 (9%)	2/34 (6%)
First incidence (days)	527	—	665	730 (T)
Poly-3 test	P=0.450	P=0.226N	P=0.358	P=0.690N
Clitoral Gland: Adenoma				
Overall rate	10/49 (20%)	12/50 (24%)	8/49 (16%)	10/49 (20%)
Adjusted rate	22.1%	26.0%	18.0%	22.7%
Terminal rate	8/33 (24%)	10/39 (26%)	7/34 (21%)	8/33 (24%)
First incidence (days)	497	656	665	529
Poly-3 test	P=0.517N	P=0.422	P=0.411N	P=0.574
Clitoral Gland: Carcinoma				
Overall rate	2/49 (4%)	3/50 (6%)	2/49 (4%)	0/49 (0%)
Adjusted rate	4.5%	6.5%	4.5%	0.0%
Terminal rate	1/33 (3%)	2/39 (5%)	1/34 (3%)	0/33 (0%)
First incidence (days)	704	634	665	—
Poly-3 test	P=0.127N	P=0.520	P=0.690N	P=0.243N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	12/49 (24%)	15/50 (30%)	9/49 (18%)	10/49 (20%)
Adjusted rate	26.4%	32.3%	20.2%	22.7%
Terminal rate	9/33 (27%)	12/39 (31%)	8/34 (24%)	8/33 (24%)
First incidence (days)	497	634	665	529
Poly-3 test	P=0.275N	P=0.349	P=0.326N	P=0.433N
Mammary Gland: Fibroadenoma				
Overall rate	23/50 (46%)	18/50 (36%)	20/50 (40%)	17/50 (34%)
Adjusted rate	49.1%	38.7%	42.8%	36.9%
Terminal rate	14/33 (42%)	15/39 (39%)	15/35 (43%)	11/34 (32%)
First incidence (days)	596	634	592	529
Poly-3 test	P=0.220N	P=0.210N	P=0.343N	P=0.162N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	23/50 (46%)	18/50 (36%)	20/50 (40%)	18/50 (36%)
Adjusted rate	49.1%	38.7%	42.8%	39.1%
Terminal rate	14/33 (42%)	15/39 (39%)	15/35 (43%)	12/34 (35%)
First incidence (days)	596	634	592	529
Poly-3 test	P=0.300N	P=0.210N	P=0.343N	P=0.221N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	15/49 (31%)	21/50 (42%)	25/49 (51%)	25/49 (51%)
Adjusted rate	32.4%	44.2%	54.9%	54.2%
Terminal rate	8/33 (24%)	16/39 (41%)	20/35 (57%)	15/33 (46%)
First incidence (days)	497	600	648	590
Poly-3 test	P=0.042	P=0.166	P=0.022	P=0.025
Thyroid Gland (C-Cell): Adenoma				
Overall rate	4/49 (8%)	6/50 (12%)	4/50 (8%)	3/50 (6%)
Adjusted rate	9.0%	13.0%	8.7%	6.8%
Terminal rate	3/33 (9%)	5/39 (13%)	3/35 (9%)	3/34 (9%)
First incidence (days)	527	634	592	730 (T)
Poly-3 test	P=0.315N	P=0.390	P=0.630N	P=0.508N
Thyroid Gland (C-Cell): Carcinoma				
Overall rate	2/49 (4%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate	4.5%	4.4%	0.0%	6.8%
Terminal rate	2/33 (6%)	2/39 (5%)	0/35 (0%)	1/34 (3%)
First incidence (days)	730 (T)	730 (T)	—	646
Poly-3 test	P=0.358	P=0.680N	P=0.231N	P=0.504
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	6/49 (12%)	8/50 (16%)	4/50 (8%)	6/50 (12%)
Adjusted rate	13.4%	17.4%	8.7%	13.5%
Terminal rate	5/33 (15%)	7/39 (18%)	3/35 (9%)	4/34 (12%)
First incidence (days)	527	634	592	646
Poly-3 test	P=0.489N	P=0.411	P=0.354N	P=0.617
Uterus: Stromal Polyp				
Overall rate	10/50 (20%)	8/50 (16%)	7/50 (14%)	10/50 (20%)
Adjusted rate	22.0%	17.5%	15.1%	22.5%
Terminal rate	5/33 (15%)	8/39 (21%)	4/35 (11%)	9/34 (27%)
First incidence (days)	630	730 (T)	527	592
Poly-3 test	P=0.425	P=0.393N	P=0.285N	P=0.576
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	11/50 (22%)	8/50 (16%)	7/50 (14%)	10/50 (20%)
Adjusted rate	24.1%	17.5%	15.1%	22.5%
Terminal rate	5/33 (15%)	8/39 (21%)	4/35 (11%)	9/34 (27%)
First incidence (days)	630	730 (T)	527	592
Poly-3 test	P=0.494	P=0.300N	P=0.205N	P=0.525N
Zymbal's Gland: Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	6.5%
Terminal rate	0/33 (0%)	0/39 (0%)	0/35 (0%)	0/34 (0%)
First incidence (days)	—	—	—	360
Poly-3 test	P=0.008	—[f]	—	P=0.125

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
All Organs: Mononuclear Cell Leukemia				
Overall rate	14/50 (28%)	17/50 (34%)	16/50 (32%)	3/50 (6%)
Adjusted rate	30.1%	35.6%	33.5%	6.7%
Terminal rate	7/33 (21%)	12/39 (31%)	9/35 (26%)	1/34 (3%)
First incidence (days)	448	575	527	603
Poly-3 test	P<0.001N	P=0.361	P=0.445	P=0.003N
All Organs: Benign Neoplasms				
Overall rate	41/50 (82%)	39/50 (78%)	41/50 (82%)	37/50 (74%)
Adjusted rate	83.5%	81.8%	85.2%	77.5%
Terminal rate	26/33 (79%)	32/39 (82%)	31/35 (89%)	26/34 (77%)
First incidence (days)	497	600	527	529
Poly-3 test	P=0.260N	P=0.520N	P=0.515	P=0.313N
All Organs: Malignant Neoplasms				
Overall rate	22/50 (44%)	24/50 (48%)	22/50 (44%)	15/50 (30%)
Adjusted rate	46.1%	48.4%	44.9%	31.4%
Terminal rate	12/33 (36%)	15/39 (39%)	12/35 (34%)	5/34 (15%)
First incidence (days)	448	396	232	360
Poly-3 test	P=0.047N	P=0.491	P=0.535N	P=0.101N
All Organs: Benign or Malignant Neoplasms				
Overall rate	45/50 (90%)	47/50 (94%)	48/50 (96%)	43/50 (86%)
Adjusted rate	90.0%	94.0%	96.0%	86.0%
Terminal rate	28/33 (85%)	36/39 (92%)	33/35 (94%)	27/34 (79%)
First incidence (days)	448	396	232	360
Poly-3 test	P=0.159N	P=0.357	P=0.217	P=0.380N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, pituitary gland, 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 are the P values 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**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE B4a
Historical Incidence of Zymbal's Gland Carcinoma in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Southern Research Institute	
2,2-Bis(bromomethyl)-1,3-propanediol	0/50
Benzyl acetate	1/50
Butyl benzyl phthalate	0/50
D&C Yellow No. 11	0/50
o-Nitroanisole	1/50
p-Nitrobenzoic acid	0/50
Overall Historical Incidence	
Total (%)	5/901 (0.6%)
Mean ± standard deviation	0.6% ± 1.2%
Range	0%-4%

[a] Data as of 10 November 1998

TABLE B4b
Historical Incidence of Mononuclear Cell Leukemia in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Southern Research Institute	
2,2-Bis(bromomethyl)-1,3-propanediol	15/50
Benzyl acetate	9/50
Butyl benzyl phthalate	21/50
D&C Yellow No. 11	16/50
o-Nitroanisole	14/50
p-Nitrobenzoic acid	17/50
Overall Historical Incidence	
Total (%)	261/901 (29.0%)
Mean ± standard deviation	29.0% ± 7.8%
Range	16%-42%

[a] Data as of 10 November 1998; includes data for lymphocytic, monocytic, and undifferentiated leukemia

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

	0 ppm	280 ppm	830 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	65	65	65	65
6-Month interim evaluation	10	10	10	10
12-Month interim evaluation	5	5	5	5
Early deaths				
Moribund	12	8	11	15
Natural deaths	5	3	4	1
Survivors				
Terminal sacrifice	33	39	35	34
Animals examined microscopically	65	65	65	65
6-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Basophilic focus	2 (20%)		1 (10%)	1 (10%)
Infiltration cellular, mixed cell		1 (10%)		
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Nephropathy	1 (10%)	2 (20%)	1 (10%)	3 (30%)
Renal tubule, hyaline droplet	6 (60%)	10 (100%)	10 (100%)	10 (100%)
Renal tubule, pigmentation	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Systems Examined with No Lesions Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
12-Month Interim Evaluation				
Alimentary System				
Liver	(5)	(5)	(5)	(5)
Basophilic focus	4 (80%)	1 (20%)	2 (40%)	4 (80%)
Infiltration cellular, mixed cell		1 (20%)	1 (20%)	1 (20%)
Bile duct, hyperplasia	1 (20%)			

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

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
12-Month Interim Evaluation (continued)				
Urinary System				
Kidney	(5)	(5)	(5)	(5)
Nephropathy	3 (60%)	3 (60%)	4 (80%)	4 (80%)
Renal tubule, hyaline droplet	3 (60%)	5 (100%)	5 (100%)	5 (100%)
Renal tubule, pigmentation	5 (100%)	5 (100%)	5 (100%	5 (100%)
Systems Examined with No Lesions Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
2-Year Study				
Alimentary System				
Intestine large, cecum	(49)	(50)	(49)	(50)
Edema			1 (2%)	
Hemorrhage			1 (2%)	
Inflammation, acute			1 (2%)	
Intestine small, duodenum	(48)	(50)	(49)	(50)
Epithelium, hyperplasia			1 (2%)	
Liver	(49)	(50)	(50)	(50)
Angiectasis			2 (4%)	4 (8%)
Basophilic focus	39 (80%)	44 (88%)	41 (82%)	45 (90%)
Clear cell focus	2 (4%)	10 (20%)	4 (8%)	11 (22%)
Eosinophilic focus	18 (37%)	10 (20%)	18 (36%)	27 (54%)
Hematopoietic cell proliferation	2 (4%)		2 (4%)	2 (4%)
Hemorrhage	1 (2%)		1 (2%)	
Hepatodiaphragmatic nodule	3 (6%)	9 (18%)	7 (14%)	7 (14%)
Infiltration cellular, mixed cell	15 (31%)	12 (24%)	9 (18%)	11 (22%)
Mixed cell focus	4 (8%)	5 (10%)	6 (12%)	1 (2%)
Necrosis, focal	4 (8%)	4 (8%)	3 (6%)	
Bile duct, hyperplasia	1 (2%)		4 (8%)	1 (2%)
Centrilobular, necrosis		1 (2%)		1 (2%)
Hepatocyte, vacuolization cytoplasmic	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Kupffer cell, pigmentation	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Mesentery	(1)	(6)	(1)	(6)
Accessory spleen		2 (33%)		2 (33%)
Fat, necrosis	1 (100%)	4 (67%)	1 (100%)	5 (83%)
Pancreas	(49)	(50)	(49)	(50)
Atrophy	13 (27%)	15 (30%)	18 (37%)	13 (26%)
Metaplasia, hepatocyte	1 (2%)			
Salivary glands	(49)	(50)	(50)	(50)
Atrophy	1 (2%)		1 (2%)	2 (4%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Stomach, forestomach	(49)	(50)	(50)	(50)
Edema	1 (2%)	1 (2%)	1 (2%)	
Erosion			1 (2%)	
Perforation			1 (2%)	
Epithelium, hyperplasia	1 (2%)			
Stomach, glandular	(49)	(50)	(50)	(50)
Edema			1 (2%)	
Erosion		1 (2%)	1 (2%)	1 (2%)
Tongue		(1)		
Epithelium, hyperplasia		1 (100%)		
Cardiovascular System				
Heart	(49)	(50)	(50)	(50)
Cardiomyopathy	22 (45%)	16 (32%)	17 (34%)	16 (32%)
Inflammation, chronic active		1 (2%)		
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Accessory adrenal cortical nodule	6 (12%)	4 (8%)	7 (14%)	10 (20%)
Atrophy				2 (4%)
Degeneration, fatty	12 (24%)	9 (18%)	14 (28%)	10 (20%)
Hemorrhage	1 (2%)	1 (2%)		
Hyperplasia, diffuse	1 (2%)			
Hyperplasia, focal	3 (6%)	3 (6%)	8 (16%)	7 (14%)
Hypertrophy	1 (2%)			
Hypertrophy, focal	12 (24%)	7 (14%)	12 (24%)	10 (20%)
Necrosis		2 (4%)		
Adrenal medulla	(47)	(50)	(50)	(50)
Hyperplasia	2 (4%)	2 (4%)	2 (4%)	3 (6%)
Islets, pancreatic	(49)	(50)	(49)	(50)
Hyperplasia		1 (2%)		
Pituitary gland	(49)	(50)	(49)	(49)
Pars distalis, angiectasis	9 (18%)	10 (20%)	6 (12%)	5 (10%)
Pars distalis, cyst	22 (45%)	11 (22%)	13 (27%)	16 (33%)
Pars distalis, hyperplasia, focal	13 (27%)	10 (20%)	3 (6%)	5 (10%)
Pars intermedia, cyst		1 (2%)		
Thyroid gland	(49)	(50)	(50)	(50)
Ultimobranchial cyst	1 (2%)		1 (2%)	
C-cell, hyperplasia	21 (43%)	9 (18%)	11 (22%)	12 (24%)
Follicle, cyst	1 (2%)	1 (2%)	2 (4%)	2 (4%)
General Body System				
None				
Genital System				
Clitoral gland	(49)	(50)	(49)	(49)
Cyst	1 (2%)		4 (8%)	1 (2%)
Hyperplasia	3 (6%)	2 (4%)	6 (12%)	3 (6%)
Inflammation, chronic	5 (10%)	5 (10%)	6 (12%)	5 (10%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Genital System (continued)				
Ovary	(49)	(50)	(50)	(50)
Cyst	10 (20%)	6 (12%)	6 (12%)	7 (14%)
Uterus	(49)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Hydrometra	5 (10%)	3 (6%)	5 (10%)	6 (12%)
Hyperplasia, cystic	1 (2%)	5 (10%)	4 (8%)	7 (14%)
Inflammation, chronic	1 (2%)		1 (2%)	2 (4%)
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Hyperplasia	5 (10%)	3 (6%)	4 (8%)	6 (12%)
Infiltration cellular, histiocyte		2 (4%)	4 (8%)	2 (4%)
Lymph node	(5)	(3)	(4)	(3)
Mediastinal, hemorrhage			2 (50%)	1 (33%)
Mediastinal, hyperplasia, lymphoid			1 (25%)	1 (33%)
Mediastinal, pigmentation	1 (20%)	1 (33%)	1 (25%)	
Pancreatic, hematopoietic cell proliferation			1 (25%)	
Pancreatic, hemorrhage			1 (25%)	
Lymph node, mandibular	(49)	(50)	(50)	(50)
Ectasia	1 (2%)	2 (4%)	6 (12%)	6 (12%)
Hemorrhage	2 (4%)	4 (8%)	4 (8%)	4 (8%)
Hyperplasia, lymphoid	1 (2%)		2 (4%)	1 (2%)
Pigmentation	18 (37%)	19 (38%)	21 (42%)	25 (50%)
Lymph node, mesenteric	(49)	(50)	(49)	(50)
Hemorrhage	2 (4%)	5 (10%)	2 (4%)	4 (8%)
Pigmentation	2 (4%)	2 (4%)		2 (4%)
Spleen	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Depletion cellular		1 (2%)		
Developmental malformation				1 (2%)
Fibrosis				2 (4%)
Hematopoietic cell proliferation	27 (54%)	24 (48%)	31 (62%)	40 (80%)
Hemorrhage	1 (2%)		1 (2%)	
Hyperplasia, histiocytic			1 (2%)	
Necrosis	1 (2%)	1 (2%)		
Pigmentation	30 (60%)	30 (60%)	35 (70%)	43 (86%)
Thymus	(47)	(49)	(49)	(50)
Cyst				1 (2%)
Integumentary System				
Mammary gland	(49)	(50)	(50)	(50)
Hyperplasia	44 (90%)	44 (88%)	42 (84%)	42 (84%)
Skin	(49)	(50)	(50)	(50)
Hyperkeratosis	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Inflammation, chronic	1 (2%)		1 (2%)	
Ulcer	2 (4%)	1 (2%)	4 (8%)	3 (6%)
Epidermis, hyperplasia	4 (8%)	2 (4%)	5 (10%)	5 (10%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Emodin

	0 ppm	280 ppm	830 ppm	2,500 ppm
2-Year Study (continued)				
Musculoskeletal System				
Bone	(49)	(50)	(50)	(50)
Cranium, osteopetrosis	7 (14%)	5 (10%)	6 (12%)	8 (16%)
Femur, osteopetrosis	6 (12%)	8 (16%)	7 (14%)	7 (14%)
Skeletal muscle			(1)	
Atrophy			1 (100%)	
Nervous System				
Brain	(49)	(50)	(50)	(50)
Compression	8 (16%)	7 (14%)	10 (20%)	7 (14%)
Hydrocephalus	3 (6%)		3 (6%)	7 (14%)
Peripheral nerve	(2)	(1)	(3)	
Atrophy			1 (33%)	
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Hemorrhage	3 (6%)	1 (2%)	2 (4%)	5 (10%)
Infiltration cellular, histiocyte	38 (78%)	37 (74%)	34 (68%)	32 (64%)
Inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	
Alveolar epithelium, hyperplasia	3 (6%)	4 (8%)		4 (8%)
Nose	(49)	(50)	(50)	(50)
Foreign body	1 (2%)		1 (2%)	2 (4%)
Inflammation, chronic	6 (12%)	3 (6%)	5 (10%)	5 (10%)
Goblet cell, hyperplasia	1 (2%)		1 (2%)	
Respiratory epithelium, hyperplasia	6 (12%)	4 (8%)	4 (8%)	1 (2%)
Respiratory epithelium, metaplasia, squamous	1 (2%)	1 (2%)		2 (4%)
Special Senses System				
Eye	(5)	(2)	(4)	(2)
Cataract	4 (80%)	2 (100%)	3 (75%)	1 (50%)
Hemorrhage			2 (50%)	
Inflammation, chronic			1 (25%)	1 (50%)
Retina, degeneration	4 (80%)	2 (100%)	3 (75%)	1 (50%)
Urinary System				
Kidney	(49)	(50)	(49)	(50)
Cyst			1 (2%)	
Glomerulosclerosis			1 (2%)	
Hydronephrosis			1 (2%)	
Infarct			2 (4%)	3 (6%)
Inflammation, chronic		1 (2%)	1 (2%)	3 (6%)
Nephropathy	46 (94%)	43 (86%)	45 (92%)	49 (98%)
Papilla, necrosis			1 (2%)	
Renal tubule, hyaline droplet	22 (45%)	49 (98%)	49 (100%)	50 (100%)
Renal tubule, hyperplasia, oncocytic				2 (4%)
Renal tubule, necrosis	1 (2%)			
Renal tubule, pigmentation	45 (92%)	49 (98%)	49 (100%)	50 (100%)
Transitional epithelium, hyperplasia	2 (4%)		1 (2%)	1 (2%)
Urinary bladder	(49)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Necrosis			1 (2%)	

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF EMODIN

TABLE C1 Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Emodin 135
TABLE C2 Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin 140
TABLE C3 Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Emodin 158
TABLE C4a Historical Incidence of Renal Tubule Neoplasms in Untreated Male $B6C3F_1$ Mice 162
TABLE C4b Historical Incidence of Malignant Lymphoma in Untreated Male $B6C3F_1$ Mice 162
TABLE C5 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Emodin 163

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

	0 ppm	160 ppm	312 ppm	625 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
12-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	4	6	6	7
Natural deaths	5	7	4	
Survivors				
Terminal sacrifice	41	37	40	43
Animals examined microscopically	60	60	60	60
12-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hepatocellular carcinoma	1 (10%)			1 (10%)
Hepatocellular adenoma		1 (10%)	2 (20%)	
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Gallbladder	(48)	(46)	(50)	(49)
Adenoma	1 (2%)			
Intestine large, cecum	(47)	(49)	(50)	(50)
Carcinoma			1 (2%)	
Intestine small, duodenum	(47)	(49)	(50)	(50)
Polyp adenomatous			1 (2%)	
Intestine small, jejunum	(47)	(46)	(49)	(50)
Carcinoma			4 (8%)	
Leiomyosarcoma	1 (2%)		1 (2%)	
Intestine small, ileum	(48)	(49)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Hemangiosarcoma	2 (4%)		1 (2%)	1 (2%)
Hemangiosarcoma, multiple	1 (2%)			
Hepatoblastoma	1 (2%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Liver (continued)	(50)	(50)	(50)	(50)
Hepatocellular carcinoma	13 (26%)	15 (30%)	17 (34%)	12 (24%)
Hepatocellular carcinoma, multiple	7 (14%)	10 (20%)	5 (10%)	4 (8%)
Hepatocellular adenoma	7 (14%)	5 (10%)	5 (10%)	7 (14%)
Hepatocellular adenoma, multiple	2 (4%)	5 (10%)	7 (14%)	4 (8%)
Leiomyosarcoma, metastatic, intestine small, jejunum			1 (2%)	
Mesentery	(2)	(4)	(6)	(5)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (25%)		
Carcinoma, metastatic, pancreas				1 (20%)
Hepatocellular carcinoma, metastatic, liver			1 (17%)	1 (20%)
Leiomyosarcoma, metastatic, intestine small, jejunum			1 (17%)	
Pancreas	(49)	(50)	(50)	(50)
Hemangioma			1 (2%)	
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Leiomyosarcoma, metastatic, intestine small, jejunum			1 (2%)	
Duct, carcinoma				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)			
Squamous cell papilloma	1 (100%)			
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	
Capsule, adenoma	1 (2%)	5 (10%)	4 (8%)	2 (4%)
Adrenal medulla	(50)	(49)	(50)	(50)
Pheochromocytoma benign	1 (2%)	1 (2%)		
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma		1 (2%)		
Parathyroid gland	(44)	(47)	(46)	(45)
Carcinoma				1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	1 (2%)	4 (8%)		
Follicular cell, adenoma, multiple		1 (2%)		
Follicular cell, carcinoma	1 (2%)			
General Body System				
None				

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
2-Year Study (continued)				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Mast cell tumor malignant	1 (2%)			
Lymph node	(6)	(3)	(4)	(1)
Mediastinal, hepatocellular carcinoma, metastatic, liver		1 (33%)		
Pancreatic, hepatocellular carcinoma, metastatic, liver	1 (17%)			
Lymph node, mandibular	(49)	(49)	(50)	(48)
Lymph node, mesenteric	(49)	(46)	(50)	(49)
Spleen	(50)	(50)	(50)	(49)
Hemangiosarcoma			1 (2%)	1 (2%)
Thymus	(42)	(45)	(46)	(39)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Mast cell tumor malignant	1 (2%)			
Pinna, fibrosarcoma	1 (2%)			
Sebaceous gland, adenoma	1 (2%)			
Subcutaneous tissue, hemangioma			1 (2%)	
Subcutaneous tissue, hemangiosarcoma		1 (2%)		1 (2%)
Subcutaneous tissue, hepatocellular carcinoma, metastatic, liver			1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)			
Musculoskeletal System				
Skeletal muscle		(3)	(1)	(2)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (33%)		
Carcinoma, metastatic, pancreas				1 (50%)
Hemangiosarcoma, metastatic, liver				1 (50%)
Leiomyosarcoma, metastatic, intestine small, jejunum			1 (100%)	
Nervous System				
Brain	(49)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	9 (18%)	9 (18%)	5 (10%)	7 (14%)
Alveolar/bronchiolar adenoma, multiple			1 (2%)	
Alveolar/bronchiolar carcinoma	9 (18%)	6 (12%)	6 (12%)	5 (10%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	3 (6%)		
Carcinoma, metastatic, pancreas				1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
2-Year Study (continued)				
Respiratory System (continued)				
Lung (continued)	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver	2 (4%)	3 (6%)	5 (10%)	3 (6%)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)			
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Mediastinum, leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Ear		(1)		
External ear, histiocytic sarcoma		1 (100%)		
Harderian gland	(4)	(3)	(4)	(7)
Adenoma	4 (100%)	2 (67%)	3 (75%)	4 (57%)
Carcinoma		1 (33%)	1 (25%)	3 (43%)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Carcinoma, metastatic, pancreas				1 (2%)
Renal tubule, adenoma		1 (2%)	1 (2%)	
Renal tubule, carcinoma			1 (2%)	1 (2%)
Urethra		(1)		
Urinary bladder	(49)	(50)	(50)	(50)
Hemangioma	1 (2%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Lymphoma malignant	5 (10%)	3 (6%)	3 (6%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]				
12-Month interim evaluation	1	1	2	1
2-Year study	43	46	43	37
Total primary neoplasms				
12-Month interim evaluation	1	1	2	1
2-Year study	74	76	71	56
Total animals with benign neoplasms				
12-Month interim evaluation		1	2	
2-Year study	24	27	24	22
Total benign neoplasms				
12-Month interim evaluation		1	2	
2-Year study	29	36	30	24
Total animals with malignant neoplasms				
12-Month interim evaluation	1			1
2-Year study	31	33	32	26
Total malignant neoplasms				
12-Month interim evaluation	1			1
2-Year study	45	40	41	32
Total animals with metastatic neoplasms				
2-Year study	3	4	6	5
Total metastatic neoplasms				
2-Year study	5	11	11	10

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	556	636	636	639	639	656	680	699	728	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	004	023	051	026	056	060	041	046	027	005	007	011	012	013	014	017	020	024	025	028	029	036	042	048	054
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Intestine large, colon	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	A	+	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma				X																					
Intestine small, ileum	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hemangiosarcoma, multiple	X																								
Hepatoblastoma																									
Hepatocellular carcinoma		X	X					X							X		X			X				X	
Hepatocellular carcinoma, multiple				X					X				X												
Hepatocellular adenoma										X					X			X							
Hepatocellular adenoma, multiple																									
Mesentery																									
Pancreas	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																+									
Squamous cell papilloma																X									
Tooth							+										+		+						+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign			X																						
Islets, pancreatic	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	M	+	M	M	+	M	+	I	+	+	+	M	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma														X											
Follicular cell, carcinoma											X														
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	
Carcass ID Number	058	059	002	003	006	008	016	018	032	034	035	037	039	047	052	053	055	057	009	015	019	030	040	044	045	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma						X																				1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leiomyosarcoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma															X				X							2
Hemangiosarcoma, multiple																										1
Hepatoblastoma											X															1
Hepatocellular carcinoma												X	X		X					X		X		X		13
Hepatocellular carcinoma, multiple							X	X													X		X			7
Hepatocellular adenoma					X	X											X		X							7
Hepatocellular adenoma, multiple									X									X								2
Mesentery						+														+						2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell papilloma																										1
Tooth	+			+							+						+	+	+	+					+	12
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma		X																								1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	M	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	44
Pituitary gland	+	+	+	+	M	+	M	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	40
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
Follicular cell, carcinoma																										1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	556	636	636	639	639	656	680	699	728	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	004	023	051	026	056	060	041	046	027	005	007	011	012	013	014	017	020	024	025	028	029	036	042	048	054
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant																									
Lymph node		+	+	+												+									
Pancreatic, hepatocellular carcinoma, metastatic, liver				X																					
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	I	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant																									
Pinna, fibrosarcoma																									
Sebaceous gland, adenoma																									
Subcutaneous tissue, schwannoma, malignant						X																			
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X			X				X										
Alveolar/bronchiolar carcinoma							X	X									X		X	X					
Alveolar/bronchiolar carcinoma, multiple																									
Hepatocellular carcinoma, metastatic, liver		X						X																	
Leiomyosarcoma, metastatic, intestine small, jejunum				X																					
Mediastinum, leiomyosarcoma, metastatic, intestine small, jejunum				X																					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland					+								+							+					
Adenoma					X								X							X					
Urinary System																									
Kidney	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant															X	X									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	
Carcass ID Number	058	059	002	003	006	008	016	018	032	034	035	037	039	047	052	053	055	057	059	015	019	030	040	044	045	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mast cell tumor malignant				X																						1
Lymph node							+				+															6
Pancreatic, hepatocellular carcinoma, metastatic, liver																										1
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	M	+	+	+	+	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	42
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mast cell tumor malignant																					X					1
Pinna, fibrosarcoma						X																				1
Sebaceous gland, adenoma																X										1
Subcutaneous tissue, schwannoma, malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X		X				X			X								X			X					9
Alveolar/bronchiolar carcinoma				X							X	X				X										9
Alveolar/bronchiolar carcinoma, multiple																						X				1
Hepatocellular carcinoma, metastatic, liver																										2
Leiomyosarcoma, metastatic, intestine small, jejunum																										1
Mediastinum, leiomyosarcoma, metastatic, intestine small, jejunum																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland																					+					4
Adenoma																					X					4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangioma																X										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant						X	X				X															5

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 160 ppm

Number of Days on Study	446	461	554	631	632	636	639	645	677	695	699	705	715	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	083	073	094	093	118	091	066	067	071	081	111	110	109	062	068	076	077	080	084	085	088	094	096	097	099
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung								X																	
Hepatocellular carcinoma	X	X	X		X	X	X		X														X	X	X
Hepatocellular carcinoma, multiple								X		X		X		X	X				X						
Hepatocellular adenoma																X									
Hepatocellular adenoma, multiple																						X			
Mesentery								+													+				
Alveolar/bronchiolar carcinoma, metastatic, lung								X																	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth											+						+		+	+	+	+			
Cardiovascular System																									
Blood vessel							+																		
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung								X																	
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Capsule, adenoma																X					X		X		
Adrenal medulla	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X														
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+
Pituitary gland	+	M	+	I	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	M	+	+	M	M
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																X									
Follicular cell, adenoma, multiple																									
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 160 ppm

Number of Days on Study	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	
Carcass ID Number	101	112	113	114	115	120	063	064	065	069	072	074	087	089	090	092	098	103	105	107	075	081	100	102	117	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hepatocellular carcinoma						X							X		X						X			X		15
Hepatocellular carcinoma, multiple	X															X		X	X							10
Hepatocellular adenoma			X			X																X		X		5
Hepatocellular adenoma, multiple					X									X	X		X									5
Mesentery											+	+														4
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma														X												1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth		+	+		+	+	+	+			+	+			+					+			+			17
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma											X															1
Capsule, adenoma							X		X																	5
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign								X																		1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	47
Pituitary gland	+	M	+	+	+	I	+	I	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	38
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma				X						X							X									4
Follicular cell, adenoma, multiple																				X						1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 160 ppm

Number of Days on Study	446	461	554	631	632	636	639	645	677	695	699	705	715	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	083	073	094	093	098	091	096	096	067	071	081	111	110	109	062	068	076	077	080	084	085	088	094	096	097	099
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+												+								
Mediastinal, hepatocellular carcinoma, metastatic, liver						X																				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	M	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	I	+	+	M	+	+	M	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung								X																		
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma																	X									
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle								+						+												+
Alveolar/bronchiolar carcinoma, metastatic, lung								X																		
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X							X	X						X		X				
Alveolar/bronchiolar carcinoma					X									X				X							X	
Alveolar/bronchiolar carcinoma, multiple								X																		
Hepatocellular carcinoma, metastatic, liver					X	X																				
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung								X																		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Ear																										
External ear, histiocytic sarcoma																										
Harderian gland													+													
Adenoma																										
Carcinoma													X													
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung								X																		
Renal tubule, adenoma																	X									
Urethra																										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																										
Lymphoma malignant																					X					

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 160 ppm

Number of Days on Study	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	
Carcass ID Number	101	112	113	114	115	120	063	064	065	069	072	074	087	089	090	092	098	103	105	107	075	081	100	102	117	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node											+															3
Mediastinal, hepatocellular carcinoma, metastatic, liver																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										3
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma												X	X				X		X							9
Alveolar/bronchiolar carcinoma																				X				X		6
Alveolar/bronchiolar carcinoma, multiple									X																X	3
Hepatocellular carcinoma, metastatic, liver	X																									3
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear													+													1
External ear, histiocytic sarcoma													X													1
Harderian gland												+							+							3
Adenoma												X							X							2
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Renal tubule, adenoma																										1
Urethra											+															1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma													X													1
Lymphoma malignant											X					X										3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	497	541	572	579	581	639	688	688	726	727	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	134	132	160	124	127	174	151	170	162	147	121	123	128	129	133	135	136	141	146	148	149	150	153	158	165
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																			X						
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous										X															
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																	X								
Leiomyosarcoma			X																						
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																		X							
Hepatocellular carcinoma	X	X	X		X		X	X						X	X					X			X		
Hepatocellular carcinoma, multiple				X		X			X	X												X			
Hepatocellular adenoma	X											X	X		X				X						
Hepatocellular adenoma, multiple																								X	
Leiomyosarcoma, metastatic, intestine small, jejunum			X																						
Mesentery			+	+										+									+		
Hepatocellular carcinoma, metastatic, liver				X																					
Leiomyosarcoma, metastatic, intestine small, jejunum			X																						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma	X																								
Leiomyosarcoma, metastatic, intestine small, jejunum			X																						
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth								+		+						+		+	+			+		+	
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Capsule, adenoma																					X				
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	I	+	+	+	I	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
Tissue NOS				+								+													

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	729	729	729	729	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	172	173	178	179	152	157	167	168	171	175	177	122	126	130	131	137	139	143	145	155	156	159	163	166	180	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp adenomatous																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma	X			X																			X			4
Leiomyosarcoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Hepatocellular carcinoma		X			X									X						X			X	X	X	17
Hepatocellular carcinoma, multiple																										5
Hepatocellular adenoma																										5
Hepatocellular adenoma, multiple								X		X	X	X					X								X	7
Leiomyosarcoma, metastatic, intestine small, jejunum																										1
Mesentery	+	+																								6
Hepatocellular carcinoma, metastatic, liver																										1
Leiomyosarcoma, metastatic, intestine small, jejunum																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Leiomyosarcoma, metastatic, intestine small, jejunum																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth					+	+	+			+		+			+	+	+	+	+		+					18
Cardiovascular System																										
Blood vessel												+														1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																						X				1
Capsule, adenoma					X													X		X						4
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	46
Pituitary gland	M	+	+	M	+	I	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
Tissue NOS																										2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	497	541	572	579	581	639	688	688	726	727	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	134	132	160	124	127	174	151	170	162	147	121	123	128	129	133	135	136	141	146	148	149	150	153	158	165
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node										+										+					
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																			X						
Thymus	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangioma																									
Subcutaneous tissue, hepatocellular Carcinoma, metastatic, liver				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+																						
Leiomyosarcoma, metastatic, intestine small, jejunum			X																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+																								
Spinal cord	+																								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma			X													X									
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma								X					X								X				
Hepatocellular carcinoma, metastatic, liver				X		X	X		X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland																									+
Adenoma																									
Carcinoma																									X
Zymbal's gland																									+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	729	729	729	729	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	172	173	178	179	152	157	167	168	171	175	177	122	126	130	131	137	139	143	145	155	156	159	163	166	180	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node					+											+										4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangioma															X											1
Subcutaneous tissue, hepatocellular Carcinoma, metastatic, liver																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Leiomyosarcoma, metastatic, intestine small, jejunum																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma								X								X		X								5
Alveolar/bronchiolar adenoma, multiple														X												1
Alveolar/bronchiolar carcinoma					X						X														X	6
Hepatocellular carcinoma, metastatic, liver																								X		5
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland			+					+												+						4
Adenoma			X					X												X						3
Carcinoma																										1
Zymbal's gland																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	497	541	572	579	581	639	688	688	726	727	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	134	132	160	124	127	174	151	170	162	147	121	123	128	129	133	135	136	141	146	148	149	150	153	158	165
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																										
Renal tubule, carcinoma																						X			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant										X				X										X	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	729	729	729	729	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	172	173	178	179	152	157	167	168	171	175	177	122	126	130	131	137	139	143	145	155	156	159	163	166	180	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																X										1
Renal tubule, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																										3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 625 ppm

Number of Days on Study	281	604	632	633	684	723	725	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	213	201	215	227	202	224	238	187	190	195	198	207	210	211	218	220	221	223	228	229	231	234	235	236	239
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma		X																							
Hepatocellular carcinoma				X	X				X		X				X		X					X			X
Hepatocellular carcinoma, multiple			X									X						X							
Hepatocellular adenoma																	X	X	X						X
Hepatocellular adenoma, multiple																								X	
Mesentery			+								+							+							
Carcinoma, metastatic, pancreas			X																						
Hepatocellular carcinoma, metastatic, liver																		X							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver			X																						
Duct, carcinoma			X																						
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth								+		+		+		+	+									+	
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																				X					
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma	X																								
Pituitary gland	+	+	+	+	M	+	M	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 625 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	
Carcass ID Number	181	185	186	188	193	194	196	203	204	212	214	216	217	219	222	225	226	232	182	189	191	205	209	233	240	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Hepatocellular carcinoma																		X	X	X			X			12
Hepatocellular carcinoma, multiple										X																4
Hepatocellular adenoma	X			X													X									7
Hepatocellular adenoma, multiple						X				X								X								4
Mesentery																							+		+	5
Carcinoma, metastatic, pancreas																										1
Hepatocellular carcinoma, metastatic, liver																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic, liver																										1
Duct, carcinoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																			X							1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+			+					+																+	10
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma												X														2
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	M	I	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	45
Carcinoma																										1
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 625 ppm

Number of Days on Study	281	604	632	633	684	723	725	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	213	201	215	227	202	224	238	187	190	195	198	207	210	211	218	220	221	223	228	229	231	234	235	236	239
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node											+														
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+
Lymph node, mesenteric	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma						X																			
Thymus	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	M
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma						X																			
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle		+	+																						
Carcinoma, metastatic, pancreas			X																						
Hemangiosarcoma, metastatic, liver		X																							
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve			+			+	+																		
Spinal cord			+			+	+																		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma													X										X		
Alveolar/bronchiolar carcinoma														X						X			X	X	
Carcinoma, metastatic, pancreas			X																						
Hepatocellular carcinoma, metastatic, liver			X	X																					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																		+							
Harderian gland																		+							
Adenoma																									
Carcinoma																		X							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pancreas			X																						
Renal tubule, carcinoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant											X														

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Emodin: 625 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	
Carcass ID Number	181	185	186	188	193	194	196	203	204	212	214	216	217	219	222	225	226	232	182	189	191	205	209	233	240	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Thymus	+	M	+	+	+	M	+	+	I	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	39
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Carcinoma, metastatic, pancreas																										1
Hemangiosarcoma, metastatic, liver																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										3
Spinal cord																										3
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma							X		X			X	X	X												7
Alveolar/bronchiolar carcinoma														X												5
Carcinoma, metastatic, pancreas																										1
Hepatocellular carcinoma, metastatic, liver										X																3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																								+		2
Harderian gland			+		+	+	+										+							+		7
Adenoma			X		X												X							X		4
Carcinoma						X	X																			3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, pancreas																										1
Renal tubule, carcinoma							X																			1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant																										1

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	1/50 (2%)	6/50 (12%)	5/50 (10%)	2/50 (4%)
Adjusted rate[b]	2.1%	13.1%	10.7%	4.2%
Terminal rate[c]	1/41 (2%)	6/37 (16%)	5/40 (13%)	2/43 (5%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test[d]	P=0.538N	P=0.050	P=0.097	P=0.501
Harderian Gland: Adenoma				
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)	4/50 (8%)
Adjusted rate	8.4%	4.4%	6.4%	8.4%
Terminal rate	3/41 (7%)	2/37 (5%)	3/40 (8%)	4/43 (9%)
First incidence (days)	639	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.492	P=0.359N	P=0.515N	P=0.641
Harderian Gland: Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	2.2%	2.1%	6.3%
Terminal rate	0/41 (0%)	0/37 (0%)	1/40 (3%)	3/43 (7%)
First incidence (days)	—[e]	715	729 (T)	729 (T)
Poly-3 test	P=0.055	P=0.492	P=0.496	P=0.120
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	4/50 (8%)	7/50 (14%)
Adjusted rate	8.4%	6.6%	8.6%	14.7%
Terminal rate	3/41 (7%)	2/37 (5%)	4/40 (10%)	7/43 (16%)
First incidence (days)	639	715	729 (T)	729 (T)
Poly-3 test	P=0.144	P=0.525N	P=0.628	P=0.260
Liver: Hemangiosarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.2%	0.0%	2.1%	2.1%
Terminal rate	2/41 (5%)	0/37 (0%)	1/40 (3%)	0/43 (0%)
First incidence (days)	556	—	729 (T)	604
Poly-3 test	P=0.284N	P=0.128N	P=0.317N	P=0.305N
Liver: Hepatocellular Adenoma				
Overall rate	9/50 (18%)	10/50 (20%)	12/50 (24%)	11/50 (22%)
Adjusted rate	18.9%	21.9%	25.4%	23.1%
Terminal rate	9/41 (22%)	10/37 (27%)	11/40 (28%)	11/43 (26%)
First incidence (days)	729 (T)	729 (T)	497	729 (T)
Poly-3 test	P=0.357	P=0.461	P=0.305	P=0.405
Liver: Hepatocellular Carcinoma				
Overall rate	20/50 (40%)	25/50 (50%)	22/50 (44%)	16/50 (32%)
Adjusted rate	41.1%	50.5%	44.0%	32.9%
Terminal rate	15/41 (37%)	15/37 (41%)	12/40 (30%)	13/43 (30%)
First incidence (days)	636	446	497	632
Poly-3 test	P=0.141N	P=0.231	P=0.465	P=0.267N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	28/50 (56%)	32/50 (64%)	31/50 (62%)	22/50 (44%)
Adjusted rate	57.5%	64.7%	62.0%	45.3%
Terminal rate	23/41 (56%)	22/37 (60%)	21/40 (53%)	19/43 (44%)
First incidence (days)	636	446	497	632
Poly-3 test	P=0.078N	P=0.302	P=0.403	P=0.156N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	21/50 (42%)	25/50 (50%)	22/50 (44%)	16/50 (32%)
Adjusted rate	43.2%	50.5%	44.0%	32.9%
Terminal rate	16/41 (39%)	15/37 (41%)	12/40 (30%)	13/43 (30%)
First incidence (days)	636	446	497	632
Poly-3 test	P=0.106N	P=0.299	P=0.547	P=0.204N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	29/50 (58%)	32/50 (64%)	31/50 (62%)	22/50 (44%)
Adjusted rate	59.6%	64.7%	62.0%	45.3%
Terminal rate	24/41 (59%)	22/37 (60%)	21/40 (53%)	19/43 (44%)
First incidence (days)	636	446	497	632
Poly-3 test	P=0.055N	P=0.378	P=0.485	P=0.111N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	9/50 (18%)	9/50 (18%)	6/50 (12%)	7/50 (14%)
Adjusted rate	18.9%	19.5%	12.7%	14.7%
Terminal rate	8/41 (20%)	7/37 (19%)	5/40 (13%)	7/43 (16%)
First incidence (days)	699	636	572	729 (T)
Poly-3 test	P=0.281N	P=0.571	P=0.296N	P=0.392N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	10/50 (20%)	9/50 (18%)	6/50 (12%)	5/50 (10%)
Adjusted rate	20.9%	19.4%	12.8%	10.5%
Terminal rate	8/41 (20%)	7/37 (19%)	5/40 (13%)	5/43 (12%)
First incidence (days)	680	632	688	729 (T)
Poly-3 test	P=0.078N	P=0.532N	P=0.221N	P=0.131N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	18/50 (36%)	17/50 (34%)	12/50 (24%)	10/50 (20%)
Adjusted rate	37.6%	36.4%	25.4%	21.0%
Terminal rate	16/41 (39%)	13/37 (35%)	10/40 (25%)	10/43 (23%)
First incidence (days)	680	632	572	729 (T)
Poly-3 test	P=0.027N	P=0.536N	P=0.143N	P=0.057N
Small Intestine (Jejunum): Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	8.6%	0.0%
Terminal rate	0/41 (0%)	0/37 (0%)	4/40 (10%)	0/43 (0%)
First incidence (days)	—	—	729 (T)	—
Poly-3 test	P=0.518	—[f]	P=0.058	—

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	1/50 (2%)	5/50 (10%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.1%	11.0%	0.0%	0.0%
Terminal rate	1/41 (2%)	5/37 (14%)	0/40 (0%)	0/43 (0%)
First incidence (days)	729 (T)	729 (T)	—	—
Poly-3 test	P=0.106N	P=0.092	P=0.504N	P=0.499N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	0/50 (0%)	0/50 (0%)
Adjusted rate	4.2%	11.0%	0.0%	0.0%
Terminal rate	2/41 (5%)	5/37 (14%)	0/40 (0%)	0/43 (0%)
First incidence (days)	729 (T)	729 (T)	—	—
Poly-3 test	P=0.052N	P=0.200	P=0.242N	P=0.236N
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	2/50 (4%)
Adjusted rate	6.2%	2.2%	4.3%	4.2%
Terminal rate	2/41 (5%)	1/37 (3%)	2/40 (5%)	0/43 (0%)
First incidence (days)	556	729 (T)	729 (T)	604
Poly-3 test	P=0.488N	P=0.324N	P=0.515N	P=0.499N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	4/50 (8%)	1/50 (2%)	4/50 (8%)	2/50 (4%)
Adjusted rate	8.3%	2.2%	8.5%	4.2%
Terminal rate	3/41 (7%)	1/37 (3%)	3/40 (8%)	0/43 (0%)
First incidence (days)	556	729 (T)	497	604
Poly-3 test	P=0.379N	P=0.195N	P=0.633	P=0.337N
All Organs: Malignant Lymphoma				
Overall rate	5/50 (10%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	10.5%	6.6%	6.4%	2.1%
Terminal rate	5/41 (12%)	3/37 (8%)	2/40 (5%)	1/43 (2%)
First incidence (days)	729 (T)	729 (T)	727	729 (T)
Poly-3 test	P=0.076N	P=0.379N	P=0.368N	P=0.101N
All Organs: Benign Neoplasms				
Overall rate	24/50 (48%)	27/50 (54%)	24/50 (48%)	22/50 (44%)
Adjusted rate	49.7%	58.5%	50.3%	46.1%
Terminal rate	21/41 (51%)	24/37 (65%)	21/40 (53%)	22/43 (51%)
First incidence (days)	636	636	497	729 (T)
Poly-3 test	P=0.289N	P=0.254	P=0.559	P=0.442N
All Organs: Malignant Neoplasms				
Overall rate	31/50 (62%)	33/50 (66%)	32/50 (64%)	26/50 (52%)
Adjusted rate	62.4%	66.6%	64.0%	52.0%
Terminal rate	23/41 (56%)	22/37 (60%)	22/40 (55%)	20/43 (47%)
First incidence (days)	556	446	497	281
Poly-3 test	P=0.119N	P=0.410	P=0.517	P=0.200N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	43/50 (86%)	46/50 (92%)	43/50 (86%)	37/50 (74%)
Adjusted rate	86.0%	92.7%	86.0%	74.0%
Terminal rate	34/41 (83%)	34/37 (92%)	33/40 (83%)	31/43 (72%)
First incidence (days)	556	446	497	281
Poly-3 test	P=0.023N	P=0.225	P=0.612	P=0.106N

(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, 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 are the P values 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**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE C4a
Historical Incidence of Renal Tubule Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
2,2-Bis(bromomethyl)-1,3-propanediol	0/49	0/49	0/49
Benzyl acetate	0/50	0/50	0/50
o-Nitroanisole	1/50	0/50	1/50
p-Nitrobenzoic acid	0/50	0/50	0/50
t-Butylhydroquinone	0/50	0/50	0/50
Overall Historical Incidence			
Total (%)	1/851 (0.1%)	1/851 (0.1%)	2/851 (0.2%)
Mean ± standard deviation	0.1% ± 0.5%	0.1% ± 0.5%	0.2% ± 0.7%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of 3 November 1998

TABLE C4b
Historical Incidence of Malignant Lymphoma in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls
Historical Incidence at Southern Research Institute	
2,2-Bis(bromomethyl)-1,3-propanediol	2/50
Benzyl acetate	2/50
o-Nitroanisole	4/50
p-Nitrobenzoic acid	2/50
t-Butylhydroquinone	4/50
Overall Historical Incidence	
Total (%)	63/852 (7.4%)
Mean ± standard deviation	7.4% ± 3.9%
Range	2%-14%

[a] Data as of 3 November 1998; includes data for histiocytic, lymphocytic, mixed, unspecified, or undifferentiated cell types

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

	0 ppm	160 ppm	312 ppm	625 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
12-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	4	6	6	7
Natural deaths	5	7	4	
Survivors				
Terminal sacrifice	41	37	40	43
Animals examined microscopically	60	60	60	60
12-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Inflammation, focal	5 (50%)	4 (40%)	2 (20%)	6 (60%)
Musculoskeletal System				
Bone			(1)	
Hyperostosis			1 (100%)	
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Cyst	1 (10%)	1 (10%)		
Mineralization, focal	1 (10%)	1 (10%)		
Nephropathy	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Renal tubule, dilation		1 (10%)	2 (20%)	
Renal tubule, pigmentation		7 (70%)	10 (100%)	10 (100%)
Systems Examined with No Lesions Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Nervous System				
Respiratory System				
Special Senses System				
2-Year Study				
Alimentary System				
Gallbladder	(48)	(46)	(50)	(49)
Cyst	1 (2%)			
Inflammation, chronic			1 (2%)	
Epithelium, hyperplasia			1 (2%)	

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

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Intestine small, jejunum	(47)	(46)	(49)	(50)
Inflammation, focal				2 (4%)
Ulcer				1 (2%)
Peyer's patch, hyperplasia, lymphoid		1 (2%)		
Intestine small, ileum	(48)	(49)	(50)	(50)
Inflammation, focal	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Basophilic focus	2 (4%)			2 (4%)
Clear cell focus	2 (4%)	1 (2%)		4 (8%)
Congestion, focal				1 (2%)
Eosinophilic focus	7 (14%)		1 (2%)	2 (4%)
Infarct				1 (2%)
Inflammation, chronic			1 (2%)	
Inflammation, focal	1 (2%)			
Mixed cell focus	1 (2%)			
Necrosis, focal		4 (8%)	2 (4%)	2 (4%)
Centrilobular, vacuolization cytoplasmic		1 (2%)		
Hepatocyte, fatty change, diffuse	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hepatocyte, vacuolization cytoplasmic, focal	13 (26%)	10 (20%)	7 (14%)	6 (12%)
Mesentery	(2)	(4)	(6)	(5)
Inflammation, chronic			1 (17%)	1 (20%)
Fat, necrosis	2 (100%)	2 (50%)	1 (17%)	1 (20%)
Pancreas	(49)	(50)	(50)	(50)
Atrophy, focal		1 (2%)		
Lipomatosis				1 (2%)
Lipomatosis, focal	1 (2%)			
Acinus, atrophy, focal			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Vacuolization cytoplasmic	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic		1 (2%)		1 (2%)
Ulcer			1 (2%)	
Epithelium, cyst			1 (2%)	
Epithelium, hyperplasia		1 (2%)	1 (2%)	3 (6%)
Serosa, hemorrhage				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	1 (2%)		1 (2%)	
Inflammation, focal				1 (2%)
Pigmentation, focal			1 (2%)	
Epithelium, hyperplasia			1 (2%)	1 (2%)
Glands, degeneration, cystic, focal	1 (2%)	3 (6%)	5 (10%)	1 (2%)
Glands, dysplasia, focal		1 (2%)		
Tooth	(12)	(17)	(18)	(10)
Developmental malformation	12 (100%)	16 (94%)	18 (100%)	10 (100%)
Inflammation, chronic, suppurative			2 (11%)	
Epithelium alveolus, hyperplasia		1 (6%)		
Peridontal tissue, inflammation, chronic		1 (6%)		

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
2-Year Study (continued)				
Cardiovascular System				
Blood vessel		(1)	(1)	
Inflammation, chronic		1 (100%)		
Aorta, inflammation, chronic			1 (100%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	2 (4%)	1 (2%)		1 (2%)
Cyst	1 (2%)			
Cytoplasmic alteration, focal		4 (8%)	1 (2%)	3 (6%)
Hyperplasia, focal	3 (6%)			
Hypertrophy, focal	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Capsule, hemorrhage				1 (2%)
Capsule, hyperplasia, focal	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Adrenal medulla	(50)	(49)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)		1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Parathyroid gland	(44)	(47)	(46)	(45)
Cyst	1 (2%)	4 (9%)	1 (2%)	
Pituitary gland	(40)	(38)	(42)	(46)
Pars distalis, cyst	1 (3%)	2 (5%)	1 (2%)	1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Degeneration, cystic, focal	3 (6%)	6 (12%)	8 (16%)	4 (8%)
Inflammation, chronic, focal			1 (2%)	
Follicle, cyst	2 (4%)	1 (2%)		1 (2%)
Follicular cell, hyperplasia	11 (22%)	6 (12%)	16 (32%)	18 (36%)
General Body System				
Tissue NOS			(2)	
Abdominal, hemorrhage			1 (50%)	
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Hypertrophy				1 (2%)
Inflammation, chronic			1 (2%)	
Preputial gland	(47)	(50)	(50)	(50)
Atrophy		1 (2%)		
Degeneration, cystic	15 (32%)	21 (42%)	24 (48%)	24 (48%)
Infiltration cellular, lymphocyte				1 (2%)
Inflammation, chronic	8 (17%)	6 (12%)	2 (4%)	2 (4%)
Prostate	(50)	(50)	(50)	(50)
Hemorrhage				1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation	28 (56%)	25 (50%)	18 (36%)	19 (38%)
Inflammation, chronic		1 (2%)	1 (2%)	
Testes	(50)	(50)	(50)	(50)
Hypoplasia				1 (2%)
Germinal epithelium, degeneration	1 (2%)	1 (2%)		1 (2%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
2-Year Study (continued)				
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			1 (2%)
Inflammation, chronic, focal				1 (2%)
Lymph node	(6)	(3)	(4)	(1)
Iliac, hyperplasia	1 (17%)			
Inguinal, hyperplasia, lymphoid		1 (33%)		
Inguinal, infiltration cellular, mast cell	1 (17%)			
Mediastinal, hyperplasia, lymphoid			1 (25%)	
Pancreatic, hyperplasia			1 (25%)	
Pancreatic, hyperplasia, lymphoid			1 (25%)	
Renal, hyperplasia	1 (17%)			
Lymph node, mesenteric	(49)	(46)	(50)	(49)
Angiectasis				1 (2%)
Hematopoietic cell proliferation	1 (2%)			1 (2%)
Hemorrhage	7 (14%)	4 (9%)	3 (6%)	5 (10%)
Hyperplasia	1 (2%)		1 (2%)	
Hyperplasia, histiocytic	1 (2%)			3 (6%)
Hyperplasia, lymphoid		1 (2%)	1 (2%)	2 (4%)
Spleen	(50)	(50)	(50)	(49)
Depletion cellular			1 (2%)	1 (2%)
Hematopoietic cell proliferation	13 (26%)	14 (28%)	14 (28%)	10 (20%)
Hyperplasia, lymphoid		4 (8%)	3 (6%)	2 (4%)
Thymus	(42)	(45)	(46)	(39)
Atrophy				1 (3%)
Cyst	2 (5%)	4 (9%)	3 (7%)	
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	
Subcutaneous tissue, angiectasis, focal		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis			1 (2%)	
Nervous System				
Brain	(49)	(50)	(50)	(50)
Hemorrhage, focal		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage		3 (6%)		2 (4%)
Hyperplasia, histiocytic	1 (2%)			
Infiltration cellular, mixed cell		1 (2%)		
Inflammation, chronic	1 (2%)			
Alveolar epithelium, hyperplasia	2 (4%)	1 (2%)	4 (8%)	
Bronchus, glands, cyst	1 (2%)			

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Emodin

	0 ppm	160 ppm	312 ppm	625 ppm
2-Year Study (continued)				
Respiratory System (continued)				
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative			1 (2%)	1 (2%)
Mucosa, glands, dilatation, focal	1 (2%)			
Nasolacrimal duct, cyst	1 (2%)			
Squamous epithelium, nasolacrimal duct, hyperplasia, focal			1 (2%)	1 (2%)
Special Senses System				
Eye				(2)
Atrophy				1 (50%)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Congestion				1 (2%)
Cyst		4 (8%)	3 (6%)	3 (6%)
Inflammation, suppurative			1 (2%)	
Metaplasia, focal, osseous			2 (4%)	1 (2%)
Nephropathy	49 (100%)	49 (98%)	50 (100%)	49 (98%)
Pelvis, dilatation	3 (6%)	3 (6%)	1 (2%)	6 (12%)
Renal tubule, accumulation, hyaline droplet				1 (2%)
Renal tubule, degeneration				1 (2%)
Renal tubule, dilatation				3 (6%)
Renal tubule, hyperplasia, focal	1 (2%)			
Renal tubule, pigmentation		46 (92%)	50 (100%)	50 (100%)
Renal tubule, epithelium, pigmentation			1 (2%)	
Urinary bladder	(49)	(50)	(50)	(50)
Hyperplasia, lymphoid				1 (2%)
Transitional epithelium, hyperplasia	1 (2%)		2 (4%)	

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF EMODIN

TABLE D1 Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Emodin 171

TABLE D2 Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin 176

TABLE D3 Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Emodin 198

TABLE D4 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Emodin 201

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

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
12-Month interim evaluation	10	10	10	10
Early deaths				
Accidental death			1	
Moribund	7	9	2	7
Natural deaths	6	2	7	7
Survivors				
Terminal sacrifice	37	39	40	36
Animals examined microscopically	60	60	60	60
12-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hepatocellular carcinoma		1 (10%)		
Hepatocellular adenoma	1 (10%)		1 (10%)	
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Gallbladder	(47)	(49)	(48)	(48)
Leiomyosarcoma, metastatic, uterus	1 (2%)			
Intestine large, rectum	(47)	(49)	(48)	(47)
Intestine large, cecum	(47)	(49)	(47)	(45)
Leiomyosarcoma				1 (2%)
Intestine small, jejunum	(45)	(48)	(45)	(46)
Leiomyosarcoma				1 (2%)
Intestine small, ileum	(48)	(49)	(47)	(46)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Hepatocellular carcinoma	3 (6%)	6 (12%)	8 (16%)	1 (2%)
Hepatocellular carcinoma, multiple	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Hepatocellular adenoma	8 (16%)	6 (12%)	10 (20%)	7 (14%)
Hepatocellular adenoma, multiple	1 (2%)	1 (2%)	2 (4%)	
Histiocytic sarcoma	1 (2%)	2 (4%)	4 (8%)	2 (4%)
Leiomyosarcoma, metastatic, uterus	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Mesentery	(14)	(16)	(15)	(11)
Hemangiosarcoma	1 (7%)		1 (7%)	1 (9%)
Histiocytic sarcoma	1 (7%)	2 (13%)	1 (7%)	1 (9%)
Leiomyosarcoma, metastatic, uterus	1 (7%)			
Leiomyosarcoma, metastatic, intestine small, jejunum				1 (9%)
Sarcoma	1 (7%)			
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (7%)			
Pancreas	(48)	(49)	(49)	(48)
Acinus, adenoma		1 (2%)		
Salivary glands	(50)	(50)	(50)	(49)
Histiocytic sarcoma		1 (2%)		
Stomach, forestomach	(50)	(49)	(48)	(49)
Squamous cell carcinoma	1 (2%)			
Squamous cell papilloma	1 (2%)			
Stomach, Glandular	(50)	(49)	(48)	(48)
Tooth			(2)	
Adamantinoma malignant			1 (50%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Rhabdomyosarcoma		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Histiocytic sarcoma				1 (2%)
Capsule, adenoma				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)			
Pituitary gland	(46)	(45)	(46)	(44)
Pars distalis, adenoma	4 (9%)	4 (9%)	9 (20%)	8 (18%)
Pars intermedia, adenoma				1 (2%)
Pars intermedia, carcinoma	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(48)
C-cell, adenoma			1 (2%)	
Follicular cell, adenoma	2 (4%)	5 (10%)	2 (4%)	2 (4%)
Follicular cell, adenoma, multiple				1 (2%)
General Body System				
Peritoneum	(2)			
Sarcoma, metastatic, mesentery	1 (50%)			
Tissue NOS	(2)	(3)		(1)
Abdominal, leiomyosarcoma, metastatic, intestine small, jejunum				1 (100%)
Abdominal, rhabdomyosarcoma, metastatic, heart		1 (33%)		
Abdominal, schwannoma malignant		1 (33%)		
Pelvic, fibrosarcoma	1 (50%)			
Thoracic, rhabdomyosarcoma	1 (50%)	1 (33%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
2-Year Study (continued)				
Genital System				
Ovary	(45)	(44)	(48)	(48)
Cystadenoma	1 (2%)	2 (5%)	2 (4%)	1 (2%)
Histiocytic sarcoma		2 (5%)		
Leiomyosarcoma, metastatic, uterus	1 (2%)			
Luteoma				1 (2%)
Oviduct	(1)	(1)		(1)
Uterus	(50)	(50)	(49)	(50)
Hemangioma			1 (2%)	
Hemangiosarcoma			1 (2%)	
Histiocytic sarcoma	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Leiomyoma		1 (2%)		
Leiomyosarcoma	1 (2%)			
Endometrium, polyp stromal	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Vagina		(1)		
Histiocytic sarcoma		1 (100%)		
Hematopoietic System				
Bone marrow	(48)	(50)	(49)	(50)
Hemangiosarcoma				1 (2%)
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)
Lymph node	(8)	(7)	(9)	(7)
Histiocytic sarcoma		1 (14%)		
Axillary, histiocytic sarcoma		1 (14%)		
Bronchial, histiocytic sarcoma		1 (14%)		
Deep cervical, histiocytic sarcoma		1 (14%)		
Iliac, histiocytic sarcoma		1 (14%)	2 (22%)	
Inguinal, histiocytic sarcoma		1 (14%)		
Mediastinal, histiocytic sarcoma		1 (14%)	1 (11%)	1 (14%)
Mediastinal, leiomyosarcoma, metastatic, uterus	1 (13%)			
Mediastinal, sarcoma, metastatic, peritoneum	1 (13%)			
Pancreatic, histiocytic sarcoma		1 (14%)	1 (11%)	
Popliteal, histiocytic sarcoma		1 (14%)		
Renal, histiocytic sarcoma			1 (11%)	1 (14%)
Lymph node, mandibular	(48)	(46)	(47)	(47)
Histiocytic sarcoma		2 (4%)		1 (2%)
Lymph node, mesenteric	(49)	(48)	(50)	(49)
Hemangiosarcoma				1 (2%)
Histiocytic sarcoma		2 (4%)	4 (8%)	1 (2%)
Rhabdomyosarcoma, metastatic, heart		1 (2%)		
Spleen	(48)	(49)	(49)	(49)
Hemangiosarcoma			1 (2%)	1 (2%)
Histiocytic sarcoma		2 (4%)	2 (4%)	1 (2%)
Thymus	(49)	(49)	(46)	(48)
Histiocytic Sarcoma		1 (2%)		1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
2-Year Study (continued)				
Integumentary System				
Mammary gland	(49)	(50)	(49)	(50)
Carcinoma				1 (2%)
Myoepithelioma	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)			
Sebaceous gland, carcinoma	1 (2%)			
Subcutaneous tissue, fibrosarcoma	2 (4%)		1 (2%)	
Subcutaneous tissue, hemangiosarcoma		1 (2%)		
Subcutaneous tissue, rhabdomyosarcoma, metastatic, heart		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma			1 (2%)	1 (2%)
Skeletal muscle	(2)		(2)	(3)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (33%)
Hemangiosarcoma				1 (33%)
Leiomyosarcoma, metastatic, uterus	1 (50%)			
Rhabdomyosarcoma	1 (50%)			
Nervous System				
Brain	(50)	(50)	(49)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	1 (2%)	2 (4%)	1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)		3 (6%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)		1 (2%)	1 (2%)
Carcinoma, metastatic, harderian gland				1 (2%)
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Histiocytic sarcoma		2 (4%)	1 (2%)	2 (4%)
Rhabdomyosarcoma, metastatic, heart		1 (2%)		
Schwannoma malignant, metastatic, tissue NOS		1 (2%)		
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (2%)			
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Mediastinum, histiocytic sarcoma			1 (2%)	2 (4%)
Nose	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)		
Pleura	(1)			
Sarcoma, metastatic, mesentery	1 (100%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
2-Year Study (continued)				
Special Senses System				
Harderian gland	(1)	(3)	(4)	(3)
Adenoma		3 (100%)	3 (75%)	1 (33%)
Carcinoma	1 (100%)			2 (67%)
Bilateral, carcinoma			1 (25%)	
Zymbal's gland		(1)	(1)	
Carcinoma		1 (100%)		
Urinary System				
Kidney	(49)	(50)	(50)	(49)
Histiocytic sarcoma		1 (2%)	2 (4%)	1 (2%)
Leiomyosarcoma, metastatic, uterus	1 (2%)			
Urinary bladder	(49)	(50)	(49)	(48)
Histiocytic sarcoma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	2 (4%)	5 (10%)	3 (6%)
Lymphoma malignant	9 (18%)	10 (20%)	8 (16%)	10 (20%)
Neoplasm Summary				
Total animals with primary neoplasms[c]				
12-Month interim evaluation	1	1	1	
2-Year study	34	33	43	37
Total primary neoplasms				
12-Month interim evaluation	1	1	1	
2-Year study	54	52	64	59
Total animals with benign neoplasms				
12-Month interim evaluation	1		1	
2-Year study	21	23	25	19
Total benign neoplasms				
12-Month interim evaluation	1		1	
2-Year study	25	27	34	26
Total animals with malignant neoplasms				
12-Month interim evaluation		1		
2-Year study	22	22	25	25
Total malignant neoplasms				
12-Month interim evaluation		1		
2-Year study	29	25	30	33
Total animals with metastatic neoplasms				
2-Year study	3	2		4
Total metastatic neoplasms				
2-Year study	12	5		6

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

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	484	541	548	587	592	606	608	643	645	681	684	689	721	732	732	732	732	732	732	732	732	733	733	733	733
Carcass ID Number	278	249	291	263	252	282	280	267	260	255	270	243	245	261	262	264	265	271	272	273	274	275	241	242	244
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma, metastatic, uterus											X														
Intestine large, colon	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	A	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular carcinoma, multiple											X														
Hepatocellular adenoma															X		X								
Hepatocellular adenoma, multiple														X											
Histiocytic sarcoma							X																		
Leiomyosarcoma, metastatic, uterus											X														
Mesentery				+		+	+	+			+	+				+			+		+	+			
Hemangiosarcoma																									
Histiocytic sarcoma							X																		
Leiomyosarcoma, metastatic, uterus											X														
Sarcoma												X													
Squamous cell carcinoma, metastatic, stomach, forestomach																			X						
Pancreas	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																			X						
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant									X																
Islets, pancreatic	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																	X								
Pars intermedia, carcinoma	X																								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																						X			
General Body System																									
Peritoneum												+	+												
Sarcoma, metastatic, mesentery												X													
Tissue NOS													+					+							
Pelvic, fibrosarcoma													X												
Thoracic, rhabdomyosarcoma																		X							

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

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	733	733	733	733	733	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	251	253	254	256	257	258	259	266	268	269	292	293	294	295	246	247	248	250	276	277	279	281	283	284	285	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	47
Leiomyosarcoma, metastatic, uterus																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma												X	X			X										3
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma			X						X	X					X						X			X		8
Hepatocellular adenoma, multiple																										1
Histiocytic sarcoma																										1
Leiomyosarcoma, metastatic, uterus																										1
Mesentery				+	+							+										+				14
Hemangiosarcoma				X																						1
Histiocytic sarcoma																										1
Leiomyosarcoma, metastatic, uterus																										1
Sarcoma																										1
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
Squamous cell papilloma												X														1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	M	+	I	+	+	+	+	+	+	+	M	M	+	M	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	I	+	+	+	+	+	M	+	46
Pars distalis, adenoma							X			X		X														4
Pars intermedia, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																				X						2
General Body System																										
Peritoneum																										2
Sarcoma, metastatic, mesentery																										1
Tissue NOS																										2
Pelvic, fibrosarcoma																										1
Thoracic, rhabdomyosarcoma																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	484	541	548	587	592	606	608	643	645	681	684	699	721	732	732	732	732	732	732	732	732	733	733	733	733
Carcass ID Number	278	249	291	263	252	282	280	267	260	255	270	243	245	261	262	264	265	271	272	273	274	275	241	242	244
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	A	+	A	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																								X	
Leiomyosarcoma, metastatic, uterus											X														
Oviduct												+													
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																		
Leiomyosarcoma											X														
Endometrium, polyp stromal						X																			
Hematopoietic System																									
Bone marrow	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+		+		+	+	+													
Mediastinal, leiomyosarcoma, metastatic, uterus											X														
Mediastinal, sarcoma, metastatic, peritoneum												X													
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myoepithelioma				X																					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Sebaceous gland, carcinoma																									
Subcutaneous tissue, fibrosarcoma				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle		+									+														
Leiomyosarcoma, metastatic, uterus											X														
Rhabdomyosarcoma		X																							
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+																								
Spinal cord	+																								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X		X															X			
Alveolar/bronchiolar carcinoma																									
Alveolar/bronchiolar carcinoma, multiple			X																						
Squamous cell carcinoma, metastatic, stomach, forestomach																			X						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pleura												+													
Sarcoma, metastatic, mesentery												X													
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	733	733	733	733	733	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	251	253	254	256	257	258	259	266	268	269	292	293	294	295	246	247	248	250	276	277	279	281	283	284	285	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	45
Cystadenoma																										1
Leiomyosarcoma, metastatic, uterus																										1
Oviduct																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leiomyosarcoma																										1
Endometrium, polyp stromal					X																					2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node							+								+						+					8
Mediastinal, leiomyosarcoma, metastatic, uterus																										1
Mediastinal, sarcoma, metastatic, peritoneum																										1
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	49
Myoepithelioma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma													X													1
Sebaceous gland, carcinoma																					X					1
Subcutaneous tissue, fibrosarcoma													X													2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Leiomyosarcoma, metastatic, uterus																										1
Rhabdomyosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve										+																2
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																							X			4
Alveolar/bronchiolar carcinoma												X														1
Alveolar/bronchiolar carcinoma, multiple																										1
Squamous cell carcinoma, metastatic, stomach, forestomach																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pleura																										1
Sarcoma, metastatic, mesentery																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	484	541	548	587	592	606	608	643	645	681	684	699	721	732	732	732	732	732	732	732	732	733	733	733	733
Carcass ID Number	278	249	291	263	252	282	280	267	260	255	270	243	245	261	262	264	265	271	272	273	274	275	241	242	244
Special Senses System																									
Eye																			+						
Harderian gland																			+						
Carcinoma																			X						
Urinary System																									
Kidney	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma, metastatic, uterus											X														
Urinary bladder	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma							X																		
Lymphoma malignant						X		X	X	X							X	X							

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 0 ppm

Number of Days on Study	733	733	733	733	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	251	253	254	256	257	258	259	266	268	269	292	293	294	295	246	247	248	250	276	277	279	281	283	284	285	Total Tissues/ Tumors
Special Senses System																										
Eye																										1
Harderian gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyosarcoma, metastatic, uterus																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant							X								X						X					9

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	391	461	490	527	552	633	659	681	707	720	727	732	732	732	732	732	732	733	733	733	733	733	733	733	733
Carcass ID Number	354	335	304	352	328	314	326	349	312	323	356	327	329	330	346	347	348	350	331	332	333	334	351	353	355
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma												X	X									X		X	
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma										X	X			X							X				
Hepatocellular adenoma, multiple																		X							
Histiocytic sarcoma			X			X																			
Mesentery			+			+	+		+	+	+			+	+	+		+			+			+	
Histiocytic sarcoma			X			X																			
Pancreas	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma												X													
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Stomach, forestomach	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+											+													
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhabdomyosarcoma											X														
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Pituitary gland	+	I	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pars distalis, adenoma															X										
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma												X													X
General Body System																									
Tissue NOS											+			+											
Abdominal, rhabdomyosarcoma, metastatic, heart											X														
Abdominal, schwannoma malignant																									
Thoracic, rhabdomyosarcoma														X											

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	301	302	303	305	311	313	315	316	317	318	319	320	321	322	324	325	336	337	338	339	340	357	358	359	360	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																	X						X			6
Hepatocellular carcinoma, multiple										X																1
Hepatocellular adenoma		X					X																			6
Hepatocellular adenoma, multiple																										1
Histiocytic sarcoma																										2
Mesentery		+				+								+								+				16
Histiocytic sarcoma																										2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinus, adenoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Blood vessel																	+								+	4
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Rhabdomyosarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma													X													1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	45
Pars distalis, adenoma				X				X								X										4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma										X						X		X								5
General Body System																										
Tissue NOS											+															3
Abdominal, rhabdomyosarcoma, metastatic, heart																										1
Abdominal, schwannoma malignant											X															1
Thoracic, rhabdomyosarcoma																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	391	461	490	527	552	633	659	681	707	720	727	732	732	732	732	732	732	733	733	733	733	733	733	733	733
Carcass ID Number	354	335	304	352	328	314	326	349	312	323	356	327	329	330	346	347	348	350	331	332	333	334	351	353	355
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Ovary	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma								X						X											
Histiocytic sarcoma			X			X																			
Oviduct							+																		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X			X																			
Leiomyoma																									
Endometrium, polyp stromal						X																			
Vagina			+																						
Histiocytic sarcoma			X																						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Lymph node						+	+			+						+									
Histiocytic sarcoma						X																			
Axillary, histiocytic sarcoma						X																			
Bronchial, histiocytic sarcoma						X																			
Deep cervical, histiocytic sarcoma						X																			
Iliac, histiocytic sarcoma						X																			
Inguinal, histiocytic sarcoma						X																			
Mediastinal, histiocytic sarcoma						X																			
Pancreatic, histiocytic sarcoma						X																			
Popliteal, histiocytic sarcoma						X																			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	M	+	+
Histiocytic sarcoma			X			X																			
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X			X																			
Rhabdomyosarcoma, metastatic, heart											X														
Spleen	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X			X																			
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma																									
Subcutaneous tissue, rhabdomyosarcoma, metastatic, heart											X														
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+	+					+																		
Spinal cord	+	+					+																		

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	301	302	303	305	311	313	315	316	317	318	319	320	321	322	324	325	336	337	338	339	340	357	358	359	360	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ovary	+	+	I	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	44
Cystadenoma																										2
Histiocytic sarcoma																										2
Oviduct																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Leiomyoma																								X		1
Endometrium, polyp stromal									X																	2
Vagina																										1
Histiocytic sarcoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node											+			+							+					7
Histiocytic sarcoma																										1
Axillary, histiocytic sarcoma																										1
Bronchial, histiocytic sarcoma																										1
Deep cervical, histiocytic sarcoma																										1
Iliac, histiocytic sarcoma																										1
Inguinal, histiocytic sarcoma																										1
Mediastinal, histiocytic sarcoma																										1
Pancreatic, histiocytic sarcoma																										1
Popliteal, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	46
Histiocytic sarcoma																										2
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	48
Histiocytic sarcoma																										2
Rhabdomyosarcoma, metastatic, heart																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										2
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																		X								1
Subcutaneous tissue, rhabdomyosarcoma, metastatic, heart																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										3
Spinal cord																										3

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	391	461	490	527	552	633	659	681	707	720	727	732	732	732	732	732	732	733	733	733	733	733	733	733	733
Carcass ID Number	354	335	304	352	328	314	326	349	312	323	356	327	329	330	346	347	348	350	331	332	333	334	351	353	355
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma											X														
Histiocytic sarcoma			X			X																			
Rhabdomyosarcoma, metastatic, heart											X														
Schwannoma malignant, metastatic, tissue NOS																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear	+																								
Harderian gland													+			+									
Adenoma													X			X									
Zymbal's gland				+																					
Carcinoma				X																					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X			X																			
Lymphoma malignant							X	X		X		X		X	X	X					X				

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 312 ppm

Number of Days on Study	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	301	302	303	305	311	313	315	316	317	318	319	320	321	322	324	325	336	337	338	339	340	357	358	359	360	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma								X																		1
Alveolar/bronchiolar carcinoma																										1
Histiocytic sarcoma																										2
Rhabdomyosarcoma, metastatic, heart																										1
Schwannoma malignant, metastatic, tissue NOS											X															1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Harderian gland																						+				3
Adenoma																						X				3
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymphoma malignant														X							X					10

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 625 ppm

Number of Days on Study	090	461	625	658	673	698	719	720	730	730	732	732	732	732	732	732	732	732	732	732	732	732	733	733	733
Carcass ID Number	390	416	379	395	388	363	405	362	403	417	366	367	368	369	370	401	402	404	406	407	408	409	410	361	364
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	A	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																	X		X		X				
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma											X	X						X				X	X		X
Hepatocellular adenoma, multiple																				X					
Histiocytic sarcoma		X						X	X													X			
Mesentery			+	+		+		+		+			+	+								+			+
Hemangiosarcoma			X																						
Histiocytic sarcoma																						X			
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth												+										+			
Adamantinoma malignant																						X			
Cardiovascular System																									
Blood vessel										+															+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+
Pars distalis, adenoma													X		X								X		X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																								X	
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Cystadenoma																									
Uterus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																					X				
Hemangiosarcoma					X																				
Histiocytic sarcoma																						X			
Endometrium, polyp stromal					X																				X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 625 ppm

Number of Days on Study	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	
Carcass ID Number	365	376	377	378	380	386	387	389	391	392	393	394	396	397	398	399	400	411	412	413	414	415	418	419	420	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma	X									X					X	X						X				8
Hepatocellular carcinoma, multiple							X																			1
Hepatocellular adenoma					X	X					X							X								10
Hepatocellular adenoma, multiple																								X		2
Histiocytic sarcoma																										4
Mesentery			+			+	+				+			+									+			15
Hemangiosarcoma																										1
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tooth																										2
Adamantinoma malignant																										1
Cardiovascular System																										
Blood vessel																										2
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pars distalis, adenoma	X	X		X	X													X								9
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																										1
Follicular cell, adenoma									X					X												2
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cystadenoma					X														X							2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangioma																										1
Hemangiosarcoma																										1
Histiocytic sarcoma																				X						2
Endometrium, polyp stromal																										2

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 625 ppm

Number of Days on Study	090	461	625	658	673	698	719	720	730	730	732	732	732	732	732	732	732	732	732	732	732	732	732	733	733
Carcass ID Number	390	416	379	395	388	363	405	362	403	417	366	367	368	369	370	401	402	404	406	407	408	409	410	361	364
Hematopoietic System																									
Bone marrow	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																						X			
Lymph node		+				+		+		+		+			+										
Iliac, histiocytic sarcoma		X						X																	
Mediastinal, histiocytic sarcoma								X																	
Pancreatic, histiocytic sarcoma								X																	
Renal, histiocytic sarcoma		X																							
Lymph node, mandibular	+	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X						X	X													X			
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma			X																						
Histiocytic sarcoma								X	X																
Thymus	+	M	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																									
Skeletal muscle	+					+																			
Nervous System																									
Brain	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																X									
Alveolar/bronchiolar carcinoma, multiple																									
Histiocytic sarcoma		X																							
Mediastinum, histiocytic sarcoma																						X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Special Senses System																									
Harderian gland																									
Adenoma																									
Bilateral, carcinoma																									
Zymbal's gland																	+								
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X							X																
Urinary bladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma		X						X	X													X			
Lymphoma malignant						X				X					X										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 625 ppm

Number of Days on Study	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	
Carcass ID Number	365	376	377	378	380	386	387	389	391	392	393	394	396	397	398	399	400	411	412	413	414	415	418	419	420	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Lymph node	+							+				+														9
Iliac, histiocytic sarcoma																										2
Mediastinal, histiocytic sarcoma																										1
Pancreatic, histiocytic sarcoma																										1
Renal, histiocytic sarcoma																										1
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										4
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Histiocytic sarcoma																										2
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																	X									1
Skeletal muscle																										2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X																					2
Alveolar/bronchiolar carcinoma, multiple	X																									1
Histiocytic sarcoma																										1
Mediastinum, histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Harderian gland						+									+			+							+	4
Adenoma						X									X			X								3
Bilateral, carcinoma																									X	1
Zymbal's gland																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																				X						5
Lymphoma malignant	X							X	X			X				X										8

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 1,250 ppm

Number of Days on Study	445	515	525	604	608	646	670	680	681	681	693	695	709	724	731	731	731	731	731	731	732	732	732	732	732
Carcass ID Number	464	452	463	435	425	455	436	446	456	474	440	471	457	437	447	448	449	450	458	459	460	421	422	423	424
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	A	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma														X											
Intestine small, duodenum	A	+	A	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	A	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma												X													
Intestine small, ileum	A	+	A	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hepatocellular carcinoma						X																			
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma									X				X												
Histiocytic sarcoma	X							X																	
Mesentery	+						+		+		+	+	+					+					+		
Hemangiosarcoma																									
Histiocytic sarcoma	X																								
Leiomyosarcoma, metastatic, intestine small, jejunum												X													
Pancreas	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel											+														
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Capsule, adenoma					X																				
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	M	+	+	M	+	+	+	+
Pars distalis, adenoma													X							X				X	
Pars intermedia, adenoma																									
Thyroid gland	A	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																				X					
Follicular cell, adenoma, multiple																									
General Body System																									
Tissue NOS												+													
Abdominal, leiomyosarcoma, metastatic, intestine small, jejunum												X													

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 1,250 ppm

Number of Days on Study	732	732	732	732	732	732	732	732	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	472	473	475	476	477	478	479	480	331	332	333	334	661	662	665	262	267	268	269	330	338	339	351	353	354	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	45
Leiomyosarcoma																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Leiomyosarcoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma			X																							1
Hepatocellular carcinoma																										1
Hepatocellular carcinoma, multiple														X	X										X	3
Hepatocellular adenoma		X		X																X	X	X				7
Histiocytic sarcoma																										2
Mesentery						+				+								+								11
Hemangiosarcoma																		X								1
Histiocytic sarcoma																										1
Leiomyosarcoma, metastatic, intestine small, jejunum																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																										
Blood vessel													+													2
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Capsule, adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	44
Pars distalis, adenoma			X			X	X				X					X										8
Pars intermedia, adenoma											X															1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell, adenoma		X																								2
Follicular cell, adenoma, multiple																		X								1
General Body System																										
Tissue NOS																										1
Abdominal, leiomyosarcoma, metastatic, intestine small, jejunum																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 1,250 ppm

Number of Days on Study	445	515	525	604	608	646	670	680	681	681	693	695	709	724	731	731	731	731	731	731	732	732	732	732
Carcass ID Number	464	452	463	435	425	455	436	446	456	474	440	471	457	437	447	448	449	450	458	459	460	421	422	423	424
Genital System																									
Clitoral gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									
Luteoma																									
Oviduct																		+							
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Endometrium, polyp stromal										X															
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma						X																			
Histiocytic sarcoma	X																								
Lymph node	A				+		+			+	+		+					+							
Mediastinal, histiocytic sarcoma																									
Renal, histiocytic sarcoma																									
Lymph node, mandibular	A	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Histiocytic sarcoma																									
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Histiocytic sarcoma								X																	
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Histiocytic sarcoma								X																	
Thymus	+	+	A	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma										X															
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																									
Skeletal muscle						+					+		+												
Alveolar/bronchiolar carcinoma, metastatic, lung													X												
Hemangiosarcoma						X																			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve						+																			
Spinal cord						+																			

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 1,250 ppm

Number of Days on Study	732	732	732	732	732	732	732	732	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734		
Carcass ID Number	472	473	475	476	477	478	479	480	431	432	433	434	461	462	465	426	427	428	429	430	438	439	451	453	454	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cystadenoma											X															1
Luteoma												X														1
Oviduct																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Endometrium, polyp stromal																	X									2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Histiocytic sarcoma																										1
Lymph node																		+								7
Mediastinal, histiocytic sarcoma																		X								1
Renal, histiocytic sarcoma																		X								1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																		X								1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																		X								1
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																						X				1
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																						X				1
Skeletal muscle																										3
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Hemangiosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 1,250 ppm

Number of Days on Study	445	515	525	604	608	646	670	680	681	681	693	695	709	724	731	731	731	731	731	731	732	732	732	732
Carcass ID Number	464	452	463	435	425	555	436	446	456	474	440	471	457	437	447	448	449	450	458	459	460	421	422	423	424
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X																
Alveolar/bronchiolar carcinoma																			X		X				
Alveolar/bronchiolar carcinoma, multiple													X												
Carcinoma, metastatic, harderian gland				X																					
Hepatocellular carcinoma, metastatic, liver																									
Histiocytic sarcoma	X							X																	
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung													X												
Mediastinum, histiocytic sarcoma	X							X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland				+																					
Adenoma																									
Carcinoma				X																					
Urinary System																									
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma								X																	
Urinary bladder	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X							X																	
Lymphoma malignant					X		X										X	X				X			

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Emodin: 1,250 ppm

Number of Days on Study	732	732	732	732	732	732	732	732	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	472	473	475	476	477	478	479	480	431	432	433	434	461	462	465	426	427	428	429	430	438	439	451	453	454	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Alveolar/bronchiolar carcinoma																								X		3
Alveolar/bronchiolar carcinoma, multiple																										1
Carcinoma, metastatic, harderian gland																										1
Hepatocellular carcinoma, metastatic, liver														X												1
Histiocytic sarcoma																										2
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Mediastinum, histiocytic sarcoma																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Harderian gland										+												+				3
Adenoma																						X				1
Carcinoma										X																2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																		X								3
Lymphoma malignant			X															X			X		X	X		10

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
Harderian Gland: Adenoma				
Overall rate[a]	0/50 (0%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate[b]	0.0%	6.6%	6.4%	2.2%
Terminal rate[c]	0/37 (0%)	3/39 (8%)	3/40 (8%)	1/36 (3%)
First incidence (days)	—[e]	731 (T)	731 (T)	731 (T)
Poly-3 test[d]	P=0.532	P=0.120	P=0.127	P=0.502
Harderian Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	4/50 (8%)	3/50 (6%)
Adjusted rate	2.2%	6.6%	8.5%	6.5%
Terminal rate	1/37 (3%)	3/39 (8%)	4/40 (10%)	2/36 (6%)
First incidence (days)	731 (T)	731 (T)	731 (T)	604
Poly-3 test	P=0.293	P=0.308	P=0.192	P=0.312
Liver: Hepatocellular Adenoma				
Overall rate	9/50 (18%)	7/50 (14%)	12/50 (24%)	7/50 (14%)
Adjusted rate	19.9%	15.3%	25.4%	15.3%
Terminal rate	9/37 (24%)	5/39 (13%)	12/40 (30%)	5/36 (14%)
First incidence (days)	731 (T)	720	731 (T)	681
Poly-3 test	P=0.421N	P=0.383N	P=0.351	P=0.379N
Liver: Hepatocellular Carcinoma				
Overall rate	4/50 (8%)	7/50 (14%)	9/50 (18%)	4/50 (8%)
Adjusted rate	8.8%	15.4%	19.1%	8.7%
Terminal rate	3/37 (8%)	7/39 (18%)	9/40 (23%)	3/36 (8%)
First incidence (days)	684	731 (T)	731 (T)	646
Poly-3 test	P=0.487N	P=0.263	P=0.131	P=0.637N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	13/50 (26%)	14/50 (28%)	21/50 (42%)	11/50 (22%)
Adjusted rate	28.7%	30.7%	44.5%	23.8%
Terminal rate	12/37 (32%)	12/39 (31%)	21/40 (53%)	8/36 (22%)
First incidence (days)	684	720	731 (T)	646
Poly-3 test	P=0.374N	P=0.508	P=0.083	P=0.386N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	1/50 (2%)	2/50 (4%)	1/50 (2%)
Adjusted rate	8.7%	2.2%	4.2%	2.2%
Terminal rate	2/37 (5%)	1/39 (3%)	2/40 (5%)	0/36 (0%)
First incidence (days)	592	731 (T)	731 (T)	681
Poly-3 test	P=0.163N	P=0.182N	P=0.326N	P=0.180N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	1/50 (2%)	4/50 (8%)
Adjusted rate	4.4%	2.2%	2.1%	8.8%
Terminal rate	1/37 (3%)	0/39 (0%)	1/40 (3%)	3/36 (8%)
First incidence (days)	548	727	731 (T)	709
Poly-3 test	P=0.166	P=0.501N	P=0.489N	P=0.336

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	6/50 (12%)	2/50 (4%)	3/50 (6%)	5/50 (10%)
Adjusted rate	12.9%	4.4%	6.4%	10.9%
Terminal rate	3/37 (8%)	1/39 (3%)	3/40 (8%)	3/36 (8%)
First incidence (days)	548	727	731 (T)	681
Poly-3 test	P=0.569N	P=0.140N	P=0.237N	P=0.512N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/46 (9%)	4/45 (9%)	9/46 (20%)	8/44 (18%)
Adjusted rate	9.7%	9.6%	20.7%	20.1%
Terminal rate	4/34 (12%)	4/37 (11%)	9/39 (23%)	7/32 (22%)
First incidence (days)	731 (T)	731 (T)	731 (T)	709
Poly-3 test	P=0.075	P=0.640N	P=0.133	P=0.156
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	2/50 (4%)	5/50 (10%)	2/50 (4%)	3/48 (6%)
Adjusted rate	4.4%	11.0%	4.2%	6.7%
Terminal rate	2/37 (5%)	5/39 (13%)	2/40 (5%)	3/36 (8%)
First incidence (days)	731 (T)	731 (T)	731 (T)	731 (T)
Poly-3 test	P=0.577	P=0.220	P=0.678N	P=0.494
All Organs: Hemangiosarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	2.2%	2.2%	4.2%	8.7%
Terminal rate	1/37 (3%)	1/39 (3%)	0/40 (0%)	3/36 (8%)
First incidence (days)	731 (T)	731 (T)	625	646
Poly-3 test	P=0.074	P=0.759N	P=0.520	P=0.183
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	4/50 (8%)
Adjusted rate	2.2%	2.2%	6.3%	8.7%
Terminal rate	1/37 (3%)	1/39 (3%)	1/40 (3%)	3/36 (8%)
First incidence (days)	731 (T)	731 (T)	625	646
Poly-3 test	P=0.078	P=0.759N	P=0.326	P=0.183
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	2/50 (4%)	5/50 (10%)	3/50 (6%)
Adjusted rate	2.2%	4.3%	10.4%	6.4%
Terminal rate	0/37 (0%)	0/39 (0%)	2/40 (5%)	1/36 (3%)
First incidence (days)	608	490	461	445
Poly-3 test	P=0.224	P=0.508	P=0.114	P=0.313
All Organs: Malignant Lymphoma				
Overall rate	9/50 (18%)	10/50 (20%)	8/50 (16%)	10/50 (20%)
Adjusted rate	19.4%	21.7%	16.9%	21.6%
Terminal rate	5/37 (14%)	7/39 (18%)	6/40 (15%)	8/36 (22%)
First incidence (days)	606	659	698	608
Poly-3 test	P=0.489	P=0.493	P=0.483N	P=0.496

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
All Organs: Benign Neoplasms				
Overall rate	21/50 (42%)	23/50 (46%)	25/50 (50%)	19/50 (38%)
Adjusted rate	44.7%	49.8%	52.8%	40.9%
Terminal rate	17/37 (46%)	19/39 (49%)	24/40 (60%)	15/36 (42%)
First incidence (days)	587	633	673	608
Poly-3 test	P=0.354N	P=0.387	P=0.282	P=0.434N
All Organs: Malignant Neoplasms				
Overall rate	22/50 (44%)	22/50 (44%)	25/50 (50%)	25/50 (50%)
Adjusted rate	44.4%	46.1%	51.1%	51.7%
Terminal rate	10/37 (27%)	15/39 (39%)	17/40 (43%)	15/36 (42%)
First incidence (days)	484	490	461	445
Poly-3 test	P=0.244	P=0.515	P=0.324	P=0.303
All Organs: Benign or Malignant Neoplasms				
Overall rate	34/50 (68%)	33/50 (66%)	43/50 (86%)	37/50 (74%)
Adjusted rate	68.0%	69.1%	87.8%	76.2%
Terminal rate	21/37 (57%)	26/39 (67%)	35/40 (88%)	26/36 (72%)
First incidence (days)	484	490	461	445
Poly-3 test	P=0.123	P=0.539	P=0.015	P=0.248

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values 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**.
[e] Not applicable; no neoplasms in animal group

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Emodin[a]

	0 ppm	312 ppm	625 ppm	1,250 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
12-Month interim evaluation	10	10	10	10
Early deaths				
Accidental death			1	
Moribund	7	9	2	7
Natural deaths	6	2	7	7
Survivors				
Terminal sacrifice	37	39	40	36
Animals examined microscopically	60	60	60	60
12-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Basophilic focus	2 (20%)			
Congestion, focal				1 (10%)
Inflammation, focal	10 (100%)	8 (80%)	8 (80%)	9 (90%)
Mineralization, focal			1 (10%)	
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Cyst	1 (10%)			
Nephropathy	4 (40%)	4 (40%)	5 (50%)	9 (90%)
Renal tubule, dilation		1 (10%)		
Renal tubule, pigmentation			7 (70%)	10 (100%)
Systems Examined with No Lesions Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
2-Year Study				
Alimentary System				
Gallbladder	(47)	(49)	(48)	(48)
Inflammation, chronic		1 (2%)		
Epithelium, hyperplasia		1 (2%)		
Intestine small, duodenum	(48)	(49)	(47)	(46)
Ectasia		1 (2%)		
Inflammation, chronic, focal	1 (2%)			
Necrosis, focal	1 (2%)			

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

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Intestine small, jejunum	(45)	(48)	(45)	(46)
Edema		1 (2%)		
Peyer's patch, hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)	
Intestine small, ileum	(48)	(49)	(47)	(46)
Peyer's patch, hyperplasia, lymphoid	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	2 (4%)	1 (2%)
Basophilic focus			1 (2%)	
Clear cell focus		1 (2%)		
Congestion, focal	1 (2%)		2 (4%)	1 (2%)
Cyst		1 (2%)		
Eosinophilic focus				1 (2%)
Fibrosis, focal		1 (2%)		
Hematopoietic cell proliferation	1 (2%)			1 (2%)
Hemorrhage, focal	1 (2%)		1 (2%)	2 (4%)
Hyperplasia, focal, histiocytic	1 (2%)			
Hyperplasia, focal, lymphoid			2 (4%)	
Infiltration cellular, mixed cell				1 (2%)
Inflammation, focal	5 (10%)	3 (6%)		
Mineralization, focal		1 (2%)		
Mixed cell focus	1 (2%)			
Necrosis, focal		1 (2%)		2 (4%)
Pigmentation, focal		1 (2%)		1 (2%)
Tension lipidosis				1 (2%)
Bile duct, cyst				1 (2%)
Centrilobular, vacuolization cytoplasmic	1 (2%)			1 (2%)
Hepatocyte, fatty change, diffuse	1 (2%)	3 (6%)	2 (4%)	4 (8%)
Hepatocyte, vacuolization cytoplasmic, focal			1 (2%)	
Hepatocyte, periportal, atrophy	1 (2%)	1 (2%)		
Hepatocyte, periportal, necrosis		1 (2%)		
Hepatocyte, periportal, regeneration		1 (2%)		
Hepatocyte, periportal, vacuolization cytoplasmic	1 (2%)			
Hepatocyte, centrilobular, cytomegaly				1 (2%)
Mesentery	(14)	(16)	(15)	(11)
Angiectasis		1 (6%)		
Inflammation, chronic				1 (9%)
Fat, necrosis	7 (50%)	10 (63%)	12 (80%)	6 (55%)
Pancreas	(48)	(49)	(49)	(48)
Lipomatosis	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Acinus, atrophy, diffuse			1 (2%)	
Acinus, atrophy, focal	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Acinus, focal cellular change			1 (2%)	
Duct, cyst	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Stomach, forestomach	(50)	(49)	(48)	(49)
Epithelium, hyperplasia	1 (2%)		2 (4%)	
Stomach, glandular	(50)	(49)	(48)	(48)
Erosion				1 (2%)
Epithelium, hyperplasia	1 (2%)			
Glands, degeneration, cystic, focal	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Tooth			(2)	
Developmental malformation			1 (50%)	

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
2-Year Study (continued)				
Cardiovascular System				
Blood vessel		(4)	(2)	(2)
Inflammation, chronic		1 (25%)		
Aorta, mineralization			1 (50%)	1 (50%)
Pulmonary artery, media, hyperplasia		3 (75%)		1 (50%)
Pulmonary artery, media, hypertrophy		1 (25%)	1 (50%)	
Heart	(50)	(50)	(50)	(50)
Mineralization			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)	1 (2%)	1 (2%)	
Angiectasis				1 (2%)
Cyst				2 (4%)
Cytoplasmic alteration, focal		1 (2%)	2 (4%)	
Hyperplasia, focal	1 (2%)			
Inflammation, chronic, focal				1 (2%)
Capsule, hyperplasia, focal	1 (2%)			1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Parathyroid gland	(44)	(47)	(45)	(47)
Cyst		3 (6%)		
Vacuolization cytoplasmic, focal				1 (2%)
Pituitary gland	(46)	(45)	(46)	(44)
Angiectasis	1 (2%)			1 (2%)
Pars distalis, cyst		1 (2%)	1 (2%)	
Pars distalis, cytoplasmic alteration, focal	2 (4%)	2 (4%)	3 (7%)	3 (7%)
Pars distalis, hyperplasia, focal	3 (7%)	1 (2%)	3 (7%)	3 (7%)
Thyroid gland	(50)	(50)	(50)	(48)
Cyst				1 (2%)
Degeneration, cystic, focal	14 (28%)	1 (2%)	5 (10%)	9 (19%)
Inflammation, chronic, focal	2 (4%)		1 (2%)	
C-cell, hyperplasia				1 (2%)
Follicle, cyst			1 (2%)	
Follicular cell, hyperplasia	9 (18%)	11 (22%)	20 (40%)	8 (17%)
Follicular cell, hyperplasia, diffuse			1 (2%)	
General Body System				
Peritoneum	(2)			
Mesothelium, hyperplasia, focal	1 (50%)			
Genital System				
Clitoral gland	(49)	(48)	(50)	(49)
Degeneration, cystic	1 (2%)		1 (2%)	1 (2%)
Inflammation, chronic	1 (2%)			
Ovary	(45)	(44)	(48)	(48)
Angiectasis	3 (7%)		4 (8%)	2 (4%)
Cyst	16 (36%)	11 (25%)	11 (23%)	11 (23%)
Hemorrhage				1 (2%)
Inflammation, suppurative				1 (2%)
Thrombosis	1 (2%)			
Bilateral, cyst			1 (2%)	

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
2-Year Study (continued)				
Genital System (continued)				
Uterus	(50)	(50)	(49)	(50)
Angiectasis			1 (2%)	1 (2%)
Atrophy		1 (2%)		
Cyst				2 (4%)
Hemorrhage			1 (2%)	
Hydrometra	17 (34%)	16 (32%)	13 (27%)	13 (26%)
Inflammation, suppurative				1 (2%)
Lymphangiectasis			1 (2%)	
Cervix, hyperplasia	1 (2%)			
Endometrium, hyperplasia, cystic	47 (94%)	45 (90%)	48 (98%)	45 (90%)
Hematopoietic System				
Bone marrow	(48)	(50)	(49)	(50)
Angiectasis		1 (2%)		1 (2%)
Depletion cellular		1 (2%)		
Hyperplasia	3 (6%)	1 (2%)	1 (2%)	5 (10%)
Hyperplasia, focal, lymphoid			1 (2%)	
Lymph node	(8)	(7)	(9)	(7)
Bronchial, hyperplasia	1 (13%)			
Iliac, angiectasis				2 (29%)
Iliac, hyperplasia		1 (14%)		
Inguinal, hyperplasia, lymphoid				1 (14%)
Mediastinal, angiectasis				1 (14%)
Mediastinal, hyperplasia				1 (14%)
Pancreatic, hyperplasia, lymphoid	1 (13%)		1 (11%)	
Renal, angiectasis				1 (14%)
Renal, hyperplasia		1 (14%)		1 (14%)
Lymph node, mandibular	(48)	(46)	(47)	(47)
Angiectasis				1 (2%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Lymph node, mesenteric	(49)	(48)	(50)	(49)
Angiectasis		1 (2%)		2 (4%)
Ectasia	1 (2%)			
Hemorrhage				2 (4%)
Hyperplasia			1 (2%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Spleen	(48)	(49)	(49)	(49)
Angiectasis		1 (2%)		1 (2%)
Hematopoietic cell proliferation	9 (19%)	14 (29%)	17 (35%)	16 (33%)
Hyperplasia, lymphoid	9 (19%)	10 (20%)	10 (20%)	12 (24%)
Pigmentation, focal			1 (2%)	
Thymus	(49)	(49)	(46)	(48)
Atrophy			1 (2%)	
Cyst				1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)		
Inflammation				1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
2-Year Study (continued)				
Integumentary System (continued)				
Mammary gland	(49)	(50)	(49)	(50)
Ectasia	1 (2%)			
Hyperplasia	2 (4%)		1 (2%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Inflammation, chronic, focal		1 (2%)		
Subcutaneous tissue, edema	1 (2%)		1 (2%)	
Subcutaneous tissue, fibrosis, focal	1 (2%)		1 (2%)	1 (2%)
Subcutaneous tissue, hyperplasia, focal, mast cell		1 (2%)		
Subcutaneous tissue, inflammation, chronic, focal	1 (2%)	1 (2%)	1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy				1 (2%)
Hyperostosis	1 (2%)			
Osteoporosis		1 (2%)		
Skeletal muscle	(2)		(2)	(3)
Hemorrhage, focal			1 (50%)	
Inflammation, suppurative				1 (33%)
Nervous System				
Brain	(50)	(50)	(49)	(50)
Atrophy, focal	1 (2%)		1 (2%)	3 (6%)
Demyelination, focal		1 (2%)		
Gliosis, focal		1 (2%)		
Hemorrhage, focal		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage			2 (4%)	
Hyperplasia, lymphoid	1 (2%)	1 (2%)		
Alveolar epithelium, hyperplasia	2 (4%)	1 (2%)		1 (2%)
Bronchus, glands, cyst			1 (2%)	
Mediastinum, infiltration cellular, mixed cell				1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)			
Special Senses System				
Eye	(1)			
Cataract	1 (100%)			

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Emodin

	0 ppm	312 ppm	625 ppm	1,250 ppm
2-Year Study (continued)				
Urinary System				
Kidney	(49)	(50)	(50)	(49)
Atrophy, focal	1 (2%)			
Congestion		1 (2%)		
Metaplasia, focal, osseous		2 (4%)	2 (4%)	
Nephropathy	22 (45%)	46 (92%)	41 (82%)	48 (98%)
Pelvis, dilatation		1 (2%)		
Renal tubule, accumulation, hyaline droplet	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Renal tubule, dilatation				1 (2%)
Renal tubule, pigmentation		37 (74%)	48 (96%)	49 (100%)
Renal tubule, epithelium, pigmentation				1 (2%)
Urinary bladder	(49)	(50)	(49)	(48)
Hyperplasia, lymphoid		1 (2%)		

APPENDIX E
GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL 208
CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOL 208
RAT AND MOUSE MICRONUCLEUS TEST PROTOCOLS (INTRAPERITONEAL INJECTION) 209
MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL (FEED STUDY) 209
EVALUATION PROTOCOL 210
RESULTS 210
TABLE E1 Mutagenicity of Emodin in *Salmonella typhimurium* 211
TABLE E2 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Emodin . . . 213
TABLE E3 Induction of Micronuclei in Polychromatic Erythrocytes of Rats and Mice Treated with Emodin by Intraperitoneal Injection 214
TABLE E4 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Emodin in Feed for 14 Weeks 216

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1992). Emodin was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of emodin. The high dose was limited to 10,000 μg/plate. All positive trials were repeated under the conditions which elicited the positive response.

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.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOL

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

In the Abs test without S9, cells were incubated in McCoy's 5A medium with emodin for 10 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with emodin and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in preliminary toxicity tests.

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

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend

($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

RAT AND MOUSE MICRONUCLEUS TEST PROTOCOLS (INTRAPERITONEAL INJECTION)

Bone Marrow: Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by emodin exposure. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male F344/N rats or male or female $B6C3F_1$ mice were injected intraperitoneally [three times at 24-hour intervals] with emodin dissolved in corn oil. Solvent control animals were injected with of corn oil 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 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of five animals per dose group.

Peripheral Blood: Peripheral blood samples were obtained from male and female mice immediately prior to sacrifice after the third injection of the three-exposure protocol described above. 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 PCEs in each of five mice per dose group.

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 PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the 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 dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the 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 PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL (FEED STUDY)

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 14-week study, peripheral blood samples were obtained from male and female mice. Smears were prepared as described above for the intraperitoneal injection study and sent to SRI International for analysis. Slides were scanned to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes (NCEs) in each of 10 mice per exposure group.

The results were tabulated and the frequency of micronucleated cells among NCEs was analyzed as described above for PCEs in the intraperitoneal injection studies. Results of the 14-week study were accepted without repeat tests, because additional test data could not be obtained.

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 differing 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

Emodin, tested in two separate studies in a preincubation assay, was mutagenic in *S. typhimurium* strain TA100 in the presence of induced rat or hamster S9 liver enzymes over a concentration range of 1 to 666 μg/plate (Table E1). No mutagenicity was detected with emodin in this assay in strain TA98, with or without S9. Abs were induced in cultured CHO cells treated with 10 to 20 μg/mL emodin in the absence of S9 activation and with 100 to 200 μg/mL emodin in the presence of S9 (Table E2); the response observed without S9 was stronger than with S9. Three separate *in vivo* micronucleus tests were performed with emodin in attempts to clarify a complicated response pattern; most of the tests gave negative results. Emodin was tested for induction of micronuclei in PCEs in standard three-exposure studies; bone marrow was analyzed 24 hours after the third injection, and results in male rats and male and female mice were negative (Table E3). Peripheral blood samples from the same mice at the end of the 72-hour exposure period were also analyzed for frequency of micronuclei, and a statistically positive response was obtained for male mice only. Considering both the bone marrow and the peripheral blood data, the three-exposure micronucleus test was judged to be negative overall in male and female mice. In peripheral blood samples from mice in the 14-week feed study, an increase in the frequency of micronucleated NCEs was seen in females, but not in males (Table E4). The small increase in NCEs observed in the female mice was statistically significant ($P=0.001$), but no individual exposed group value differed significantly from the control value; the result in female mice was concluded to be weakly positive.

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

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9 Trial 1	-S9 Trial 2	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
Study 1							
TA100	0	117 ± 4.3		170 ± 4.3	130 ± 15.2	160 ± 8.5	151 ± 2.1
	3			160 ± 14.7		176 ± 6.4	165 ± 19.7
	10			174 ± 0.6	145 ± 12.0	196 ± 17.8	178 ± 13.9
	33			231 ± 23.9	182 ± 11.8	267 ± 10.7	261 ± 7.5
	66				212 ± 10.6		
	100	127 ± 2.4		232 ± 9.9	218 ± 8.0	289 ± 7.7	284 ± 14.1
	166						286 ± 19.2
	333	116 ± 1.8[d]		217 ± 21.8[d]	216 ± 5.2[d]	240 ± 29.0[d]	
	1,000	84 ± 6.1[d]					
	3,333	108 ± 6.4[d]					
	10,000	106 ± 12.2[d]					
Trial summary		Negative		Weakly positive	Weakly positive	Weakly positive	Weakly positive
Positive control[c]		336 ± 9.2		473 ± 33.1	525 ± 18.7	556 ± 19.2	562 ± 16.0
Study 2							
TA100	0	163 ± 7.5	126 ± 11.5				
	10	139 ± 4.0	124 ± 6.9				
	33	134 ± 2.0	117 ± 4.0				
	100	151 ± 1.2	91 ± 7.3				
	333	114 ± 7.8[d]	92 ± 3.8[d]				
	1,000	95 ± 6.4[d]	84 ± 8.2[d]				
Trial summary		Negative	Negative				
Positive control		977 ± 36.8	1,384 ± 50.2				
		+ hamster S9					
		5%	**10%**	**30%**	**30%**	**30%**	**30%**
TA100 (continued)	0	94 ± 3.3	95 ± 4.5	142 ± 3.0	127 ± 7.5	95 ± 9.0	117 ± 3.0
	3	113 ± 10.3	119 ± 5.4			118 ± 8.4	120 ± 14.5
	10	155 ± 6.8	146 ± 5.7	186 ± 6.3	155 ± 2.5	143 ± 3.8	140 ± 6.7
	33	150 ± 9.8	167 ± 4.8	235 ± 8.4	171 ± 8.3	175 ± 18.8	186 ± 12.3
	66	159 ± 8.7	198 ± 9.6			204 ± 14.4	223 ± 14.2
	100	139 ± 6.7	178 ± 9.8	245 ± 3.5	192 ± 12.2	239 ± 3.5	225 ± 14.7
	166	140 ± 11.1	154 ± 4.0			218 ± 16.2	
	333			235 ± 5.5[d]	169 ± 10.2[d]		
	666			187 ± 6.4[d]	152 ± 15.5[d]		
Trial summary		Equivocal	Positive	Weakly positive	Weakly positive	Positive	Positive
Positive control		1,295 ± 24.5	800 ± 43.9	950 ± 34.6	590 ± 26.3	610 ± 25.7	561 ± 38.7

TABLE E1
Mutagenicity of Emodin in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/Plate + rat S9 5%	10%	30%	30%	30%	30%
Study 2 (continued)							
TA100	0	95 ± 6.9	96 ± 10.8	162 ± 11.1	138 ± 7.2	99 ± 7.3	120 ± 4.0
(continued)	1	102 ± 8.1	106 ± 11.0	144 ± 9.0	139 ± 5.5	137 ± 10.9	136 ± 13.0
	10	143 ± 9.4	151 ± 18.4	160 ± 6.8	157 ± 4.2	139 ± 6.2	164 ± 3.1
	33	178 ± 5.7	144 ± 15.7	217 ± 4.1	211 ± 9.8	192 ± 16.9	191 ± 9.9
	66						219 ± 5.7
	100	199 ± 12.0	184 ± 12.5	234 ± 5.0	228 ± 7.4	216 ± 16.0	252 ± 19.3
	166	140 ± 5.8	141 ± 5.0			212 ± 1.7	
	333	103 ± 6.7[d]	132 ± 15.0[d]	249 ± 15.0[d]	226 ± 7.4[d]	154 ± 4.7[d]	
Trial summary		Positive	Positive	Weakly positive	Weakly positive	Positive	Positive
Positive control		740 ± 18.3	483 ± 10.9	501 ± 16.6	393 ± 22.6	474 ± 16.6	396 ± 13.3

		-S9 Trial 1	-S9 Trial 2	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
Study 1							
TA98	0	18 ± 2.5		33 ± 4.7		33 ± 2.3	
	3			32 ± 2.3		34 ± 4.4	
	10			30 ± 0.9		27 ± 3.2	
	33			41 ± 1.9		40 ± 1.2	
	100	20 ± 1.9		36 ± 4.8		33 ± 3.2	
	333	20 ± 3.4[d]		22 ± 4.5[d]		30 ± 2.2[d]	
	1,000	15 ± 1.5[d]					
	3,333	10 ± 0.6[d]					
	10,000	11 ± 2.4[d]					
Trial summary		Negative		Negative		Negative	
Positive control		636 ± 5.0		370 ± 7.5		178 ± 15.6	
Study 2							
TA98	0	27 ± 1.8	21 ± 3.5	41 ± 1.7	31 ± 0.3	39 ± 1.3	37 ± 8.3
	1					33 ± 2.0	33 ± 6.4
	10	23 ± 3.3	21 ± 4.4	39 ± 0.9	20 ± 3.2	30 ± 1.0	20 ± 0.6
	33	26 ± 3.2	24 ± 2.1	35 ± 3.8	23 ± 4.7	25 ± 3.3	24 ± 0.9
	100	29 ± 1.5	18 ± 2.8	34 ± 3.2	29 ± 3.0	28 ± 2.0	22 ± 0.9
	333	28 ± 3.3[d]	16 ± 2.9[d]	38 ± 4.5[d]	19 ± 2.0[d]	29 ± 4.1[d]	18 ± 1.8[d]
	666			32 ± 3.0[d]	16 ± 0.9[d]		
	1,000	21 ± 4.2[d]	11 ± 1.2[d]				
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		587 ± 7.5	515 ± 29.5	675 ± 19.6	482 ± 30.1	134 ± 8.7	138 ± 13.3

[a] Studies were performed at SRI International. 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] The positive controls in the absence of metabolic activation were sodium azide (TA100) and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with both strains was 2-aminoanthracene.
[d] Precipitate on plate

TABLE E2
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Emodin[a]

Compound	Dose (μg/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
-S9					
Harvest time: 12.0 hours					
Summary: Positive					
Dimethylsulfoxide[b]		200	4	0.02	2.0
Emodin	10	200	13	0.07	5.5
	15	200	25	0.13	11.5*
	20	50	17	0.34	32.0*
					P<0.001[c]
Mitomycin-C[d]	0.4	25	16	0.64	32.0*
+S9					
Harvest time: 12.0 hours					
Summary: Weakly positive					
Dimethylsulfoxide		200	2	0.01	1.0
Emodin	100	200	14	0.07	3.5
	150	200	7	0.04	3.0
	200	200	31	0.16	12.5*
					P<0.001
Cyclophosphamide[d]	20	25	6	0.24	20.0*

* Positive response (P≤0.05) versus the solvent control
[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by Galloway *et al*. (1987).
[b] Solvent control
[c] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[d] Positive control

TABLE E3
Induction of Micronuclei in Polychromatic Erythrocytes of Rats and Mice Treated with Emodin by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Bone Marrow: Micronucleated PCEs/ 1,000 PCEs[b]	Bone Marrow: Pairwise P Value[c]	Peripheral Blood: Micronucleated PCEs/ 1,000 PCEs	Peripheral Blood: Pairwise P Value
Male Rats					
Corn oil[d]		0.90 ± 0.33			
Emodin	125	0.80 ± 0.12	0.5959		
	250	1.00 ± 0.27	0.4092		
	500	0.90 ± 0.10	0.5000		
		P=0.455[e]			
Cyclophosphamide[f]	25	16.83 ± 0.67[g]	0.0000		
Male Mice					
Trial 1					
Corn oil		1.30 ± 0.37			
Emodin	125	1.50 ± 0.45	0.3526		
	250	0.60 ± 0.19	0.9459		
	500	1.30 ± 0.41	0.5000		
		P=0.626			
Cyclophosphamide	25	15.80 ± 0.98	0.0000		
Trial 2					
Corn oil		1.30 ± 0.25		1.70 ± 0.25	
Emodin	125	1.50 ± 0.27	0.3526	2.60 ± 0.75	0.1540
	250	1.63 ± 0.13[h]	0.2842	2.10 ± 0.43	0.3149
	500	1.20 ± 0.20	0.5793	3.70 ± 1.19	0.0216
		P=0.609		P=0.025	
Cyclophosphamide	25	11.20 ± 1.61	0.0000	23.88 ± 1.82[h]	0.0000

TABLE E3
Induction of Micronuclei in Polychromatic Erythrocytes of Rats and Mice Treated with Emodin by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Bone Marrow Micronucleated PCEs/ 1,000 PCEs[b]	Bone Marrow Pairwise P Value[c]	Peripheral Blood Micronucleated PCEs/ 1,000 PCEs	Peripheral Blood Pairwise P Value
Female Mice					
Corn oil		1.10 ± 0.19		1.90 ± 0.24	
Emodin	125	0.90 ± 0.37	0.6727	1.20 ± 0.41	0.8958
	250	0.60 ± 0.24	0.8875	1.10 ± 0.29	0.9281
	500	1.30 ± 0.34	0.3415	1.10 ± 0.37	0.9281
		P=0.303		P=0.915	
Cyclophosphamide	25	10.00 ± 0.82	0.0000	13.80 ± 0.46	0.0000

[a] Studies were performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al*. (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error; five animals per group had erythrocytes scored.
[c] Pairwise comparison of treated group to vehicle control. Dosed group values are significant at P≤0.008; positive control values are significant at P≤0.05 (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at P≤0.025 (ILS, 1990)
[f] Positive control
[g] n=3
[h] n=4

TABLE E4
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Emodin in Feed for 14 Weeks[a]

Compound	Dose (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	Pairwise P Value[c]
Male				
Emodin	0	10	1.23 ± 0.12	
	312.5	10	1.48 ± 0.17	0.1425
	625	10	1.32 ± 0.16	0.3391
	1,250	10	1.23 ± 0.23	0.4730
	2,500	10	1.33 ± 0.12	0.3313
	5,000	10	1.39 ± 0.20	0.2590
			P=0.406[d]	
Urethane[e]		3	16.99 ± 1.75	0.0000
Female				
Emodin	0	10	0.74 ± 0.08	
	312.5	10	0.70 ± 0.09	0.5832
	625	10	0.57 ± 0.07	0.8808
	1,250	10	0.81 ± 0.10	0.2895
	2,500	10	0.94 ± 0.10	0.1014
	5,000	10	1.06 ± 0.14	0.0250
			P=0.001	

[a] Studies were performed at SRI International. The detailed protocol is presented by MacGregor *et al*. (1990). NCE=normochromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison of treated group to control group. Exposed group values are significant at P≤0.005; positive control values are significant at P≤0.05 (ILS, 1990).
[d] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025 (ILS, 1990)
[e] Positive control; three male mice were administered urethane (0.2%) in drinking water to provide a positive control set of slides for scoring.

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1 **Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of Emodin** **218**

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of Emodin[a]

	0 ppm	312.5 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Male						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)						
Day 5	41.5 ± 0.4	42.9 ± 0.5	41.8 ± 0.3	42.8 ± 0.4*	43.0 ± 0.3*	44.5 ± 0.7**
Day 22	44.8 ± 0.4	44.2 ± 0.4	44.8 ± 0.5	44.3 ± 0.3	44.3 ± 0.6	44.3 ± 0.6
Week 14	45.6 ± 0.4	45.5 ± 0.6	45.1 ± 0.3	45.1 ± 0.5	46.5 ± 0.4	44.3 ± 0.6
Hemoglobin (g/dL)						
Day 5	13.9 ± 0.1	14.4 ± 0.2*	13.9 ± 0.1	14.3 ± 0.1	14.4 ± 0.1**	15.1 ± 0.2**
Day 22	15.5 ± 0.1	15.3 ± 0.1	15.5 ± 0.2	15.4 ± 0.1	15.3 ± 0.2	15.3 ± 0.2
Week 14	15.0 ± 0.1	14.8 ± 0.2	14.8 ± 0.1	14.8 ± 0.2	15.2 ± 0.1	14.6 ± 0.2
Erythrocytes ($10^6/\mu L$)						
Day 5	6.93 ± 0.09	7.21 ± 0.10*	6.99 ± 0.07	7.18 ± 0.08*	7.29 ± 0.06**	7.58 ± 0.11**
Day 22	7.77 ± 0.06	7.68 ± 0.08	7.74 ± 0.10	7.72 ± 0.06	7.74 ± 0.11	7.74 ± 0.11
Week 14	8.98 ± 0.08	8.89 ± 0.11	8.88 ± 0.07	8.92 ± 0.13	9.28 ± 0.06	8.89 ± 0.11
Reticulocytes ($10^6/\mu L$)						
Day 5	3.32 ± 0.28	3.41 ± 0.26	3.13 ± 0.31	3.12 ± 0.26	2.30 ± 0.20*	1.93 ± 0.13**
Day 22	3.35 ± 0.35	2.83 ± 0.20	2.81 ± 0.25	3.09 ± 0.21	3.34 ± 0.15	3.22 ± 0.26
Week 14	1.26 ± 0.18	1.45 ± 0.24	1.32 ± 0.21	1.16 ± 0.14	1.27 ± 0.20	1.74 ± 0.23
Nucleated erythrocytes ($10^3/\mu L$)						
Day 5	0.10 ± 0.02	0.06 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.07 ± 0.03	0.03 ± 0.01
Day 22	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.03	0.01 ± 0.01
Week 14	0.70 ± 0.26	0.80 ± 0.29	0.40 ± 0.16	0.70 ± 0.26	0.20 ± 0.13	0.80 ± 0.25
Mean cell volume (fL)						
Day 5	59.9 ± 0.3	59.5 ± 0.3	59.8 ± 0.2	59.7 ± 0.4	59.0 ± 0.4	58.7 ± 0.2**
Day 22	57.7 ± 0.2	57.6 ± 0.3	57.9 ± 0.4	57.3 ± 0.2	57.3 ± 0.4	57.3 ± 0.3
Week 14	50.8 ± 0.3	51.1 ± 0.4	50.8 ± 0.2	50.6 ± 0.3	50.1 ± 0.3	49.8 ± 0.3*
Mean cell hemoglobin (pg)						
Day 5	20.0 ± 0.1	19.9 ± 0.1	19.9 ± 0.1	19.9 ± 0.1	19.8 ± 0.1	19.9 ± 0.1
Day 22	20.0 ± 0.1	20.0 ± 0.1	20.0 ± 0.1	20.0 ± 0.1	19.8 ± 0.1	19.8 ± 0.1
Week 14	16.7 ± 0.1	16.6 ± 0.1	16.7 ± 0.1	16.6 ± 0.1	16.4 ± 0.1*	16.4 ± 0.1*
Mean cell hemoglobin concentration (g/dL)						
Day 5	33.4 ± 0.2	33.5 ± 0.1	33.3 ± 0.1	33.4 ± 0.1	33.6 ± 0.2	33.9 ± 0.2
Day 22	34.6 ± 0.2	34.7 ± 0.2	34.5 ± 0.3	34.9 ± 0.3	34.6 ± 0.3	34.6 ± 0.2
Week 14	32.8 ± 0.1	32.6 ± 0.2	32.8 ± 0.1	32.8 ± 0.2	32.8 ± 0.2	32.9 ± 0.1
Platelets ($10^3/\mu L$)						
Day 5	845.3 ± 16.4	846.4 ± 15.0	891.4 ± 31.8	846.1 ± 25.7	849.5 ± 27.4	893.6 ± 29.7
Day 22	754.0 ± 14.4	706.0 ± 20.6	709.1 ± 13.6	756.7 ± 12.3	769.2 ± 17.6	807.5 ± 22.4
Week 14	653.2 ± 12.2	670.3 ± 14.0	702.0 ± 19.3	687.1 ± 13.8	730.8 ± 7.3**	770.9 ± 51.2**
Leukocytes ($10^3/\mu L$)						
Day 5	3.96 ± 0.45	6.73 ± 0.70	5.53 ± 0.97	5.26 ± 0.70	4.66 ± 0.52	6.21 ± 0.80
Day 22	5.90 ± 0.61	5.29 ± 0.50	5.14 ± 0.87	5.44 ± 0.75	4.72 ± 0.80	4.73 ± 0.75
Week 14	8.65 ± 0.60	8.43 ± 0.50	9.71 ± 0.68	8.81 ± 0.56	9.92 ± 0.93	7.29 ± 0.79
Segmented neutrophils ($10^3/\mu L$)						
Day 5	0.51 ± 0.05	0.95 ± 0.11*	0.65 ± 0.10	0.69 ± 0.15	0.90 ± 0.13	1.12 ± 0.22*
Day 22	0.69 ± 0.10	0.60 ± 0.06	0.55 ± 0.09	0.65 ± 0.07	0.80 ± 0.17	0.67 ± 0.11
Week 14	1.29 ± 0.16[b]	1.42 ± 0.11	2.00 ± 0.26	1.43 ± 0.20	2.09 ± 0.38	1.91 ± 0.30
Bands ($10^3/\mu L$)						
Day 5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 22	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Lymphocytes ($10^3/\mu L$)						
Day 5	3.29 ± 0.38	5.47 ± 0.60	4.71 ± 0.86	4.31 ± 0.59	3.59 ± 0.38	4.78 ± 0.60
Day 22	4.91 ± 0.51	4.30 ± 0.42	4.33 ± 0.77	4.36 ± 0.62	3.63 ± 0.59	3.77 ± 0.58
Week 14	6.76 ± 0.34	6.80 ± 0.44	7.46 ± 0.54	7.21 ± 0.53	7.57 ± 0.69	5.17 ± 0.49

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of Emodin

	0 ppm	312.5 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Male (continued)						
n	10	10	10	10	10	10
Hematology (continued)						
Atypical lymphocytes ($10^3/\mu L$)						
Day 5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes ($10^3/\mu L$)						
Day 5	0.14 ± 0.03	0.29 ± 0.04	0.16 ± 0.05	0.26 ± 0.05	0.16 ± 0.05	0.31 ± 0.07
Day 22	0.28 ± 0.06	0.34 ± 0.06	0.22 ± 0.03	0.35 ± 0.11	0.27 ± 0.11	0.24 ± 0.07
Week 14	0.10 ± 0.04	0.15 ± 0.06	0.17 ± 0.05	0.06 ± 0.03	0.13 ± 0.06	0.13 ± 0.04
Eosinophils ($10^3/\mu L$)						
Day 5	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00
Day 22	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	0.05 ± 0.01
Week 14	0.08 ± 0.03	0.06 ± 0.02	0.08 ± 0.03	0.12 ± 0.03	0.12 ± 0.05	0.08 ± 0.02
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 5	20.3 ± 0.8	19.6 ± 0.5	18.8 ± 0.6	20.3 ± 0.3	21.8 ± 0.4	20.5 ± 0.7
Day 22	21.8 ± 0.4	22.3 ± 0.5	21.7 ± 0.5	21.1 ± 0.5	20.2 ± 0.5	22.1 ± 0.6
Week 14	22.0 ± 0.7	22.2 ± 0.7	22.4 ± 0.7	22.4 ± 0.6	22.1 ± 0.8	22.3 ± 0.7
Creatinine (mg/dL)						
Day 5	0.55 ± 0.02	0.60 ± 0.02	0.58 ± 0.03[b]	0.58 ± 0.02	0.55 ± 0.02	0.56 ± 0.03
Day 22	0.60 ± 0.02	0.59 ± 0.01[b]	0.59 ± 0.02[b]	0.59 ± 0.03[c]	0.55 ± 0.02	0.59 ± 0.01
Week 14	0.63 ± 0.02	0.70 ± 0.03	0.75 ± 0.04*	0.71 ± 0.02	0.69 ± 0.03	0.68 ± 0.03
Total protein (g/dL)						
Day 5	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.0	6.0 ± 0.1
Day 22	6.6 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.1
Week 14	6.9 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	7.3 ± 0.1*	7.1 ± 0.1
Albumin (g/dL)						
Day 5	3.4 ± 0.1	3.4 ± 0.0	3.5 ± 0.1[b]	3.4 ± 0.0	3.4 ± 0.1	3.5 ± 0.1
Day 22	3.6 ± 0.1	3.7 ± 0.1	3.6 ± 0.0	3.7 ± 0.1[b]	3.7 ± 0.0	3.7 ± 0.0
Week 14	3.8 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.9 ± 0.0	3.8 ± 0.1
Alanine aminotransferase (IU/L)						
Day 5	38 ± 1	40 ± 1	40 ± 1	41 ± 1*	43 ± 2*	44 ± 1**
Day 22	45 ± 1	39 ± 1*	41 ± 1	46 ± 6	87 ± 33	40 ± 1
Week 14	47 ± 2	45 ± 1	43 ± 2	42 ± 1	88 ± 37	103 ± 57
Alkaline phosphatase (IU/L)						
Day 5	681 ± 15	621 ± 8	633 ± 15	625 ± 23	635 ± 14	619 ± 12*
Day 22	510 ± 12	512 ± 9	491 ± 11	491 ± 11	463 ± 19	505 ± 11
Week 14	239 ± 5	249 ± 8	236 ± 8	234 ± 7	215 ± 11	225 ± 9
Creatine kinase (IU/L)						
Day 5	436 ± 58	467 ± 29[b]	500 ± 72	391 ± 35	468 ± 42	467 ± 54
Day 22	305 ± 30	341 ± 40	278 ± 23	339 ± 46	249 ± 19	334 ± 47
Week 14	353 ± 41	239 ± 43	327 ± 69	322 ± 55	310 ± 68	296 ± 54
Sorbitol dehydrogenase (IU/L)						
Day 5	6 ± 1	5 ± 1[b]	6 ± 1[b]	5 ± 1	5 ± 1[b]	6 ± 1
Day 22	9 ± 1	9 ± 1[b]	9 ± 1[b]	7 ± 1[b]	11 ± 2[b]	8 ± 1[b]
Week 14	10 ± 1	11 ± 1	10 ± 1	11 ± 1	16 ± 5	16 ± 6
Bile acids (μmol/L)						
Day 5	45.5 ± 6.6	37.8 ± 3.9[b]	37.0 ± 7.5[c]	36.4 ± 7.4[b]	43.4 ± 7.3[b]	45.1 ± 6.6[c]
Day 22	47.8 ± 3.5[b]	46.7 ± 3.7[d]	42.1 ± 6.6[b]	48.3 ± 4.3[e]	47.3 ± 4.6[b]	41.9 ± 3.7[c]
Week 14	16.7 ± 4.4	19.0 ± 2.9	19.8 ± 3.5[b]	23.4 ± 3.8	30.7 ± 4.1**	28.3 ± 4.7*

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of Emodin

	0 ppm	312.5 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Female						
Hematology						
n						
Day 8	10	10	10	10	10	10
Day 24	9	8	9	8	8	9
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 8	43.4 ± 0.4	43.6 ± 0.4	43.4 ± 0.5	43.6 ± 0.5	44.6 ± 0.7	46.4 ± 0.3**
Day 24	45.4 ± 0.5	46.0 ± 0.5	44.7 ± 0.6	44.9 ± 0.5	45.2 ± 0.6[e]	44.8 ± 0.7
Week 14	46.2 ± 0.4	46.2 ± 0.4	45.4 ± 0.3	45.4 ± 0.4	45.5 ± 0.7	45.2 ± 0.6
Hemoglobin (g/dL)						
Day 8	14.7 ± 0.1	15.0 ± 0.1	14.8 ± 0.1	14.8 ± 0.1	15.2 ± 0.3*	16.1 ± 0.1**
Day 24	15.2 ± 0.2	15.4 ± 0.2	15.1 ± 0.2	15.2 ± 0.1	15.0 ± 0.3	15.0 ± 0.2
Week 14	15.3 ± 0.1	15.1 ± 0.1	14.9 ± 0.1	14.9 ± 0.1*	14.7 ± 0.2*	14.7 ± 0.2*
Erythrocytes ($10^6/\mu L$)						
Day 8	7.48 ± 0.08	7.67 ± 0.06	7.54 ± 0.09	7.61 ± 0.09	7.71 ± 0.14	8.17 ± 0.07**
Day 24	7.88 ± 0.08	8.06 ± 0.08	7.86 ± 0.11	7.89 ± 0.07	7.84 ± 0.17	7.97 ± 0.13
Week 14	8.46 ± 0.07	8.48 ± 0.06	8.33 ± 0.07	8.39 ± 0.09	8.44 ± 0.14	8.31 ± 0.11
Reticulocytes ($10^6/\mu L$)						
Day 8	2.27 ± 0.20	2.09 ± 0.25	2.51 ± 0.32	2.22 ± 0.17	2.05 ± 0.27	1.84 ± 0.14
Day 24	1.09 ± 0.09	1.20 ± 0.15	1.26 ± 0.10	1.19 ± 0.14	1.14 ± 0.12	1.59 ± 0.23
Week 14	1.13 ± 0.25	1.38 ± 0.17	1.15 ± 0.20	1.11 ± 0.11	1.05 ± 0.17	1.40 ± 0.26
Nucleated erythrocytes ($10^3/\mu L$)						
Day 8	0.05 ± 0.02	0.07 ± 0.03	0.04 ± 0.01[b]	0.05 ± 0.02	0.03 ± 0.01	0.02 ± 0.01[b]
Day 24	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.06 ± 0.03	0.02 ± 0.01
Week 14	0.80 ± 0.29	0.70 ± 0.26	0.40 ± 0.22	0.80 ± 0.42	0.30 ± 0.21	0.30 ± 0.15
Mean cell volume (fL)						
Day 8	58.1 ± 0.3	56.9 ± 0.4	57.7 ± 0.4	57.3 ± 0.2	57.9 ± 0.3	56.8 ± 0.3
Day 24	57.7 ± 0.3	57.0 ± 0.3	56.9 ± 0.2	56.9 ± 0.2	56.6 ± 0.4*[e]	56.3 ± 0.3**
Week 14	54.7 ± 0.3	54.5 ± 0.2	54.5 ± 0.2	54.2 ± 0.2	53.9 ± 0.6	54.4 ± 0.3
Mean cell hemoglobin (pg)						
Day 8	19.6 ± 0.1	19.6 ± 0.1	19.7 ± 0.1	19.5 ± 0.1	19.7 ± 0.0	19.6 ± 0.1
Day 24	19.4 ± 0.1	19.1 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.1 ± 0.1	18.8 ± 0.1**
Week 14	18.0 ± 0.0	17.8 ± 0.1	17.9 ± 0.1	17.8 ± 0.1*	17.4 ± 0.1**	17.7 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 8	33.8 ± 0.2	34.4 ± 0.2	34.2 ± 0.2	34.1 ± 0.1	34.1 ± 0.2	34.6 ± 0.1*
Day 24	33.6 ± 0.1	33.5 ± 0.2	33.8 ± 0.1	33.8 ± 0.2	33.8 ± 0.2[e]	33.4 ± 0.2
Week 14	33.0 ± 0.2	32.8 ± 0.2	32.8 ± 0.1	32.8 ± 0.2	32.2 ± 0.2*	32.5 ± 0.1
Platelets ($10^3/\mu L$)						
Day 8	851.1 ± 17.7	790.5 ± 21.0	841.6 ± 17.7	891.0 ± 21.1	844.9 ± 26.5	821.6 ± 29.6
Day 24	688.1 ± 18.4	704.8 ± 17.4	658.2 ± 12.3	721.6 ± 22.6	713.3 ± 19.7	769.8 ± 22.6
Week 14	732.3 ± 11.5	733.9 ± 28.9	779.2 ± 60.0	734.7 ± 19.3	905.7 ± 33.5**	810.6 ± 23.9**
Leukocytes ($10^3/\mu L$)						
Day 8	4.27 ± 0.50	4.49 ± 0.26	5.12 ± 0.40	4.43 ± 0.28	5.18 ± 0.57	5.39 ± 0.51
Day 24	4.18 ± 0.32	5.35 ± 0.96	3.22 ± 0.44	2.80 ± 0.28*	4.62 ± 1.08	4.76 ± 0.62
Week 14	6.32 ± 0.33	6.10 ± 0.34	7.07 ± 0.48	6.58 ± 0.64	12.18 ± 1.03**	10.65 ± 1.61**

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of Emodin

	0 ppm	312.5 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Female (continued)						
Hematology (continued)						
n						
Day 8	10	10	10	10	10	10
Day 24	9	8	9	8	8	9
Week 14	10	10	10	10	10	10
Segmented neutrophils ($10^3/\mu L$)						
Day 8	0.55 ± 0.06	0.48 ± 0.05	0.48 ± 0.05[b]	0.52 ± 0.05	0.67 ± 0.10	0.98 ± 0.20[b]
Day 24	0.42 ± 0.05	0.54 ± 0.07	0.36 ± 0.04	0.31 ± 0.04	0.54 ± 0.11	0.85 ± 0.16
Week 14	1.11 ± 0.11	1.09 ± 0.23	1.71 ± 0.26	1.73 ± 0.33	5.27 ± 0.79**	3.90 ± 0.90**
Bands ($10^3/\mu L$)						
Day 8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[b]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[b]
Day 24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes ($10^3/\mu L$)						
Day 8	3.59 ± 0.45	3.93 ± 0.23	4.48 ± 0.42[b]	3.69 ± 0.24	4.39 ± 0.53	4.43 ± 0.30[b]
Day 24	3.66 ± 0.27	4.51 ± 0.78	2.72 ± 0.38	2.44 ± 0.26	3.91 ± 0.93	3.74 ± 0.51
Week 14	5.04 ± 0.24	4.80 ± 0.32	5.14 ± 0.32	4.65 ± 0.42	6.49 ± 0.46	6.34 ± 0.69
Atypical lymphocytes ($10^3/\mu L$)						
Day 8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[b]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[b]
Day 24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes ($10^3/\mu L$)						
Day 8	0.11 ± 0.05	0.07 ± 0.02	0.12 ± 0.03[b]	0.18 ± 0.04	0.10 ± 0.03	0.24 ± 0.07[b]
Day 24	0.06 ± 0.03	0.28 ± 0.15	0.12 ± 0.06	0.04 ± 0.02	0.15 ± 0.07	0.11 ± 0.07
Week 14	0.12 ± 0.03	0.11 ± 0.03	0.15 ± 0.03	0.17 ± 0.08	0.37 ± 0.09	0.34 ± 0.15
Eosinophils ($10^3/\mu L$)						
Day 8	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.01[b]	0.04 ± 0.02	0.02 ± 0.01	0.01 ± 0.01[b]
Day 24	0.04 ± 0.03	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.02
Week 14	0.05 ± 0.02	0.11 ± 0.02	0.08 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.06 ± 0.02
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 8	21.8 ± 0.4	21.6 ± 0.5	21.3 ± 0.4	22.5 ± 0.3	23.8 ± 0.5**	23.5 ± 0.8*
Day 24	24.2 ± 0.6	24.7 ± 0.6	23.6 ± 0.7	23.8 ± 0.7	23.7 ± 0.7	23.2 ± 0.9
Week 14	19.8 ± 0.8	21.0 ± 1.0	23.3 ± 0.8*	21.5 ± 0.7	22.9 ± 1.6	20.0 ± 1.0
Creatinine (mg/dL)						
Day 8	0.62 ± 0.02[c]	0.59 ± 0.03	0.57 ± 0.03[e]	0.60 ± 0.03	0.59 ± 0.03	0.60 ± 0.04[c]
Day 24	0.65 ± 0.01	0.66 ± 0.03[b]	0.63 ± 0.03[b]	0.63 ± 0.01[c]	0.64 ± 0.02	0.60 ± 0.02[e]
Week 14	0.73 ± 0.02	0.77 ± 0.02	0.73 ± 0.02	0.78 ± 0.03[b]	0.72 ± 0.05	0.77 ± 0.04[b]
Total protein (g/dL)						
Day 8	6.2 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.1
Day 24	6.7 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.4 ± 0.1
Week 14	7.3 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	6.8 ± 0.1**	6.6 ± 0.2**

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of Emodin

	0 ppm	312.5 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Female (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Albumin (g/dL)						
Day 8	3.7 ± 0.1	3.7 ± 0.1[b]	3.8 ± 0.0[b]	3.7 ± 0.1[c]	3.7 ± 0.1[b]	3.6 ± 0.0
Day 24	3.9 ± 0.1	3.8 ± 0.0	3.9 ± 0.1	3.8 ± 0.0	3.8 ± 0.0	3.7 ± 0.1
Week 14	4.0 ± 0.1	3.9 ± 0.0	3.9 ± 0.1	4.0 ± 0.1	3.5 ± 0.1**	3.5 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 8	35 ± 2	38 ± 1	36 ± 1	37 ± 1	37 ± 1	43 ± 2**
Day 24	39 ± 1	36 ± 1	34 ± 1*	34 ± 1	35 ± 1	38 ± 1
Week 14	35 ± 1	43 ± 2*	40 ± 2	40 ± 2	36 ± 2	43 ± 3
Alkaline phosphatase (IU/L)						
Day 8	556 ± 18	502 ± 10*	483 ± 8**	466 ± 12**	483 ± 13**	486 ± 13**
Day 24	403 ± 21	433 ± 7	392 ± 14	398 ± 10	414 ± 10	400 ± 12
Week 14	212 ± 10	222 ± 8	205 ± 6	209 ± 4	238 ± 15	198 ± 8
Creatine kinase (IU/L)						
Day 8	472 ± 67	479 ± 30	550 ± 75	440 ± 22	387 ± 32[b]	453 ± 19
Day 24	982 ± 275	527 ± 130	400 ± 49	950 ± 297	427 ± 55[b]	896 ± 227
Week 14	338 ± 69	346 ± 53	410 ± 63	310 ± 33	317 ± 43	276 ± 34
Sorbitol dehydrogenase (IU/L)						
Day 8	2 ± 1[b]	3 ± 1[e]	3 ± 1[d]	5 ± 2[c]	4 ± 1[c]	4 ± 1[e]
Day 24	5 ± 1[b]	6 ± 1[b]	6 ± 1	5 ± 1[b]	6 ± 1	4 ± 1[c]
Week 14	9 ± 1	10 ± 1	9 ± 1	9 ± 1	9 ± 1	11 ± 1
Bile acids (μmol/L)						
Day 8	33.6 ± 3.8[e]	41.0 ± 5.3[d]	18.0 ± 5.7[f]	33.9 ± 5.5[e]	37.2 ± 9.3[d]	40.3 ± 5.4[d]
Day 24	37.4 ± 4.6[c]	33.4 ± 4.1[c]	29.3 ± 3.1[b]	33.6 ± 3.9[b]	30.5 ± 6.1[d]	37.2 ± 4.4[g]
Week 14	30.6 ± 5.9[e]	17.9 ± 1.9[e]	29.9 ± 4.1[b]	21.4 ± 2.6[c]	35.1 ± 8.8[b]	21.3 ± 2.7[b]

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=8
[d] n=6
[e] n=7
[f] n=4
[g] n=5

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

TABLE G1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Feed Study of Emodin 224
TABLE G2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Feed Study of Emodin 225
TABLE G3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 12-Month Interim Evaluation in the 2-Year Feed Study of Emodin 226
TABLE G4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 16-Day Feed Study of Emodin 227
TABLE G5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Feed Study of Emodin 228
TABLE G6 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 12-Month Interim Evaluation in the 2-Year Feed Study of Emodin 229

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 16-Day Feed Study of Emodin[a]

	0 ppm	600 ppm	2,000 ppm	5,500 ppm	17,000 ppm	50,000 ppm
Male						
n	5	5	5	5	5	5
Necropsy body wt	200 ± 6	192 ± 6	201 ± 5	175 ± 5**	149 ± 6**	136 ± 6**
Heart						
Absolute	0.668 ± 0.034	0.662 ± 0.004	0.676 ± 0.024	0.598 ± 0.029	0.490 ± 0.023**	0.444 ± 0.039**
Relative	3.339 ± 0.088	3.459 ± 0.117	3.376 ± 0.120	3.416 ± 0.082	3.285 ± 0.026	3.255 ± 0.228
R. Kidney						
Absolute	0.940 ± 0.025	0.928 ± 0.021	1.054 ± 0.040	0.870 ± 0.029	0.784 ± 0.033**	0.734 ± 0.007**
Relative	4.714 ± 0.109	4.836 ± 0.088	5.256 ± 0.133	4.982 ± 0.136	5.264 ± 0.098*	5.437 ± 0.232**
Liver						
Absolute	10.194 ± 0.322	10.226 ± 0.487	11.508 ± 0.568	8.936 ± 0.299	8.144 ± 0.576*	7.328 ± 0.693**
Relative	51.078 ± 1.086	53.158 ± 1.575	57.285 ± 1.579	51.122 ± 0.686	54.393 ± 1.822	53.396 ± 2.687
Lung						
Absolute	1.008 ± 0.040	1.330 ± 0.184	1.126 ± 0.097	0.862 ± 0.020	0.740 ± 0.034	0.820 ± 0.111
Relative	5.043 ± 0.074	6.885 ± 0.903	5.614 ± 0.455	4.938 ± 0.086	4.966 ± 0.101	5.986 ± 0.697
R. Testis						
Absolute	1.084 ± 0.032	1.063 ± 0.044	1.103 ± 0.026	1.081 ± 0.033	1.022 ± 0.033	0.969 ± 0.033
Relative	5.430 ± 0.041	5.523 ± 0.084	5.506 ± 0.084	6.189 ± 0.155*	6.912 ± 0.403**	7.148 ± 0.173**
Thymus						
Absolute	0.534 ± 0.021	0.496 ± 0.021	0.517 ± 0.015	0.404 ± 0.019**	0.312 ± 0.022**	0.273 ± 0.037**
Relative	2.684 ± 0.117	2.595 ± 0.143	2.582 ± 0.063	2.322 ± 0.132	2.087 ± 0.086**	1.984 ± 0.210**
Female						
n	5	5	5	5	5	2
Necropsy body wt	143 ± 2	137 ± 4	138 ± 3	133 ± 1*	117 ± 4**	88 ± 6**
Heart						
Absolute	0.506 ± 0.012	0.514 ± 0.009	0.498 ± 0.017	0.484 ± 0.017	0.412 ± 0.015**	0.315 ± 0.025**
Relative	3.534 ± 0.089	3.752 ± 0.081	3.601 ± 0.123	3.645 ± 0.142	3.515 ± 0.120	3.581 ± 0.040
R. Kidney						
Absolute	0.654 ± 0.005	0.694 ± 0.017	0.684 ± 0.029	0.694 ± 0.006	0.642 ± 0.019	0.710 ± 0.130
Relative	4.569 ± 0.073	5.062 ± 0.092	4.933 ± 0.115	5.224 ± 0.015	5.472 ± 0.076*	8.217 ± 2.040**
Liver						
Absolute	6.220 ± 0.191	6.392 ± 0.095	6.310 ± 0.254	6.552 ± 0.136	5.980 ± 0.160	4.770 ± 0.280**
Relative	43.411 ± 1.121	46.721 ± 1.494	45.595 ± 1.624	49.313 ± 0.877**	51.020 ± 1.128**	54.302 ± 0.521**
Lung						
Absolute	0.818 ± 0.026	0.746 ± 0.033	0.776 ± 0.026	0.754 ± 0.020	0.664 ± 0.020**	0.545 ± 0.025**
Relative	5.707 ± 0.126	5.434 ± 0.191	5.609 ± 0.179	5.680 ± 0.178	5.666 ± 0.145	6.210 ± 0.139
Thymus						
Absolute	0.376 ± 0.031	0.415 ± 0.013	0.373 ± 0.019	0.367 ± 0.011	0.283 ± 0.013**	0.073 ± 0.039**
Relative	2.634 ± 0.238	3.032 ± 0.109	2.704 ± 0.177	2.765 ± 0.077	2.422 ± 0.141	0.799 ± 0.383**

* 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).

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Feed Study of Emodin[a]

	0 ppm	312.5 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	361 ± 5	363 ± 4	357 ± 8	364 ± 7	321 ± 5**	325 ± 6**
Heart						
Absolute	1.002 ± 0.020	1.000 ± 0.019	1.006 ± 0.026	1.020 ± 0.024	0.941 ± 0.018	0.916 ± 0.020**
Relative	2.773 ± 0.031	2.756 ± 0.039	2.815 ± 0.032	2.803 ± 0.039	2.931 ± 0.045*	2.819 ± 0.036
R. Kidney						
Absolute	1.236 ± 0.026	1.350 ± 0.028	1.302 ± 0.039	1.402 ± 0.025*	1.312 ± 0.029	1.349 ± 0.076
Relative	3.421 ± 0.053	3.718 ± 0.049	3.642 ± 0.062	3.859 ± 0.064**	4.087 ± 0.076**	4.146 ± 0.207**
Liver						
Absolute	12.522 ± 0.307	12.765 ± 0.261	12.738 ± 0.424	13.051 ± 0.378	11.615 ± 0.324	12.251 ± 0.359
Relative	34.630 ± 0.480	35.162 ± 0.432	35.617 ± 0.717	35.821 ± 0.563	36.133 ± 0.638	37.664 ± 0.738**
Lung						
Absolute	1.341 ± 0.025	1.343 ± 0.028	1.611 ± 0.057**	1.402 ± 0.043	1.407 ± 0.065	1.307 ± 0.030
Relative	3.713 ± 0.056	3.705 ± 0.087	4.516 ± 0.145**	3.856 ± 0.108**	4.374 ± 0.161**	4.028 ± 0.097**
R. Testis						
Absolute	1.470 ± 0.021	1.443 ± 0.031	1.453 ± 0.035[b]	1.495 ± 0.022	1.490 ± 0.030	1.456 ± 0.035
Relative	4.075 ± 0.075	3.978 ± 0.080	4.081 ± 0.060[b]	4.116 ± 0.058	4.638 ± 0.059**	4.480 ± 0.081**
Thymus						
Absolute	0.322 ± 0.023	0.307 ± 0.018	0.282 ± 0.019	0.303 ± 0.015	0.268 ± 0.013	0.260 ± 0.018
Relative	0.892 ± 0.067	0.849 ± 0.054	0.787 ± 0.045	0.833 ± 0.042	0.836 ± 0.043	0.803 ± 0.063
Female						
Necropsy body wt	196 ± 2	198 ± 4	191 ± 3	188 ± 2*	172 ± 3**	178 ± 2**
Heart						
Absolute	0.642 ± 0.016	0.619 ± 0.020	0.647 ± 0.023	0.596 ± 0.017	0.569 ± 0.017*[b]	0.602 ± 0.005
Relative	3.272 ± 0.082	3.117 ± 0.061	3.394 ± 0.094	3.173 ± 0.089	3.354 ± 0.103[b]	3.392 ± 0.045
R. Kidney						
Absolute	0.716 ± 0.011	0.710 ± 0.020	0.733 ± 0.016	0.725 ± 0.018	0.747 ± 0.017	0.809 ± 0.016**
Relative	3.649 ± 0.066	3.576 ± 0.052	3.850 ± 0.067*	3.860 ± 0.092*	4.350 ± 0.061**	4.553 ± 0.068**
Liver						
Absolute	5.958 ± 0.075	6.058 ± 0.211	6.541 ± 0.141*	5.902 ± 0.096	5.710 ± 0.112	5.988 ± 0.113
Relative	30.350 ± 0.305	30.484 ± 0.627	34.343 ± 0.475**	31.416 ± 0.415**	33.282 ± 0.565**	33.690 ± 0.378**
Lung						
Absolute	0.924 ± 0.018	0.938 ± 0.021	1.042 ± 0.030*	0.963 ± 0.048	0.897 ± 0.017[b]	0.906 ± 0.017
Relative	4.713 ± 0.117	4.729 ± 0.056	5.494 ± 0.213*	5.128 ± 0.256*	5.288 ± 0.115*[b]	5.098 ± 0.062*
Thymus						
Absolute	0.206 ± 0.005	0.211 ± 0.004	0.201 ± 0.010	0.195 ± 0.008	0.172 ± 0.006*	0.193 ± 0.010*
Relative	1.050 ± 0.032	1.066 ± 0.026	1.056 ± 0.049	1.037 ± 0.043	1.004 ± 0.033	1.085 ± 0.051

* 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 Rats at the 12-Month Interim Evaluation in the 2-Year Feed Study of Emodin[a]

	0 ppm	280 ppm	830 ppm	2,500 ppm
n	5	5	5	5
Male				
Necropsy body wt	460 ± 7	458 ± 9	435 ± 5	423 ± 15*
R. Kidney				
Absolute	1.636 ± 0.022	1.464 ± 0.045	1.538 ± 0.022	1.636 ± 0.084
Relative	3.556 ± 0.060	3.198 ± 0.077*	3.534 ± 0.037	3.864 ± 0.143
L. Kidney				
Absolute	1.672 ± 0.052	1.514 ± 0.028	1.546 ± 0.068	1.644 ± 0.063
Relative	3.630 ± 0.075	3.310 ± 0.071	3.552 ± 0.148	3.892 ± 0.137
Liver				
Absolute	17.428 ± 0.395	14.398 ± 0.299**	14.346 ± 0.510**	14.730 ± 0.508**
Relative	37.864 ± 0.700	31.498 ± 0.884**	32.929 ± 0.817**	34.812 ± 0.452*
Female				
Necropsy body wt	267 ± 9	254 ± 5	263 ± 9	257 ± 17
R. Kidney				
Absolute	0.910 ± 0.009	0.874 ± 0.036	0.864 ± 0.026	0.882 ± 0.042
Relative	3.423 ± 0.106	3.442 ± 0.081	3.302 ± 0.110	3.481 ± 0.241
L. Kidney				
Absolute	0.904 ± 0.016	0.882 ± 0.039	0.860 ± 0.022	0.876 ± 0.039
Relative	3.398 ± 0.096	3.476 ± 0.124	3.287 ± 0.101	3.460 ± 0.236
Liver				
Absolute	7.934 ± 0.333	7.264 ± 0.088	6.972 ± 0.280	8.084 ± 0.281
Relative	29.711 ± 0.535	28.694 ± 0.722	26.570 ± 0.551	31.790 ± 1.415

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-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 16-Day Feed Study of Emodin[a]

	0 ppm	600 ppm	2,000 ppm	5,500 ppm	17,000 ppm
n	5	5	5	5	5
Male					
Necropsy body wt	26.5 ± 0.5	26.0 ± 0.8	26.0 ± 0.9	24.6 ± 0.5	21.7 ± 0.5**
Heart					
Absolute	0.130 ± 0.003	0.126 ± 0.009	0.126 ± 0.005	0.120 ± 0.008	0.098 ± 0.002**
Relative	4.918 ± 0.129	4.834 ± 0.304	4.843 ± 0.124	4.882 ± 0.317	4.508 ± 0.036
R. Kidney					
Absolute	0.290 ± 0.007	0.276 ± 0.015	0.298 ± 0.012	0.264 ± 0.010	0.234 ± 0.005**
Relative	10.961 ± 0.166	10.590 ± 0.398	11.476 ± 0.473	10.727 ± 0.304	10.780 ± 0.307
Liver					
Absolute	1.452 ± 0.034	1.404 ± 0.091	1.462 ± 0.094	1.412 ± 0.050	1.254 ± 0.051
Relative	54.865 ± 0.321	53.820 ± 2.541	56.020 ± 2.189	57.352 ± 1.050	57.598 ± 1.285
Lung					
Absolute	0.168 ± 0.008	0.168 ± 0.009	0.166 ± 0.005	0.164 ± 0.007	0.142 ± 0.006
Relative	6.364 ± 0.346	6.483 ± 0.409	6.406 ± 0.285	6.681 ± 0.352	6.548 ± 0.327
R. Testis					
Absolute	0.102 ± 0.004	0.113 ± 0.005	0.107 ± 0.002	0.101 ± 0.003	0.103 ± 0.003
Relative	3.862 ± 0.114	4.384 ± 0.301	4.140 ± 0.127	4.118 ± 0.111	4.747 ± 0.165**
Thymus					
Absolute	0.048 ± 0.003	0.051 ± 0.004	0.037 ± 0.004*	0.034 ± 0.003**	0.017 ± 0.004**
Relative	1.821 ± 0.102	1.961 ± 0.117	1.418 ± 0.143	1.372 ± 0.122*	0.778 ± 0.215**
Female					
Necropsy body wt	21.5 ± 0.2	21.4 ± 0.2	21.5 ± 0.3	20.5 ± 0.7	16.7 ± 0.8**
Heart					
Absolute	0.118 ± 0.004	0.118 ± 0.002	0.106 ± 0.002*	0.100 ± 0.003**	0.088 ± 0.004**
Relative	5.506 ± 0.217	5.526 ± 0.099	4.946 ± 0.154	4.911 ± 0.256	5.283 ± 0.232
R. Kidney					
Absolute	0.190 ± 0.004	0.200 ± 0.008	0.190 ± 0.003	0.190 ± 0.010	0.190 ± 0.009
Relative	8.850 ± 0.131	9.360 ± 0.358	8.854 ± 0.038	9.267 ± 0.312	11.431 ± 0.641**
Liver					
Absolute	1.176 ± 0.025	1.200 ± 0.024	1.222 ± 0.048	1.178 ± 0.051	0.906 ± 0.061**
Relative	54.783 ± 0.800	56.173 ± 0.895	56.956 ± 2.107	57.459 ± 0.841	53.987 ± 1.785
Lung					
Absolute	0.192 ± 0.027	0.164 ± 0.008	0.156 ± 0.008	0.152 ± 0.010	0.136 ± 0.014*
Relative	8.994 ± 1.353	7.672 ± 0.339	7.297 ± 0.487	7.416 ± 0.370	8.086 ± 0.524
Thymus					
Absolute	0.065 ± 0.004	0.075 ± 0.005	0.064 ± 0.006	0.041 ± 0.005**	0.017 ± 0.007**
Relative	3.023 ± 0.182	3.498 ± 0.223	3.017 ± 0.315	2.025 ± 0.292*	0.963 ± 0.361**

* 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); no data available for 50,000 ppm group due to 100% mortality

TABLE G5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Feed Study of Emodin[a]

	0 ppm	312.5 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	37.6 ± 1.0	36.2 ± 0.5	35.3 ± 0.8	37.6 ± 0.6	33.7 ± 0.5**	33.3 ± 1.2**
Heart						
Absolute	0.151 ± 0.004	0.158 ± 0.003	0.150 ± 0.003	0.154 ± 0.003	0.151 ± 0.005	0.154 ± 0.003
Relative	4.042 ± 0.152	4.372 ± 0.077	4.259 ± 0.085	4.105 ± 0.080	4.491 ± 0.161	4.717 ± 0.300*
R. Kidney						
Absolute	0.306 ± 0.009	0.310 ± 0.004	0.301 ± 0.007	0.318 ± 0.006	0.301 ± 0.005	0.303 ± 0.006
Relative	8.187 ± 0.295	8.589 ± 0.189	8.535 ± 0.117	8.485 ± 0.215	8.953 ± 0.179*	9.210 ± 0.377**
Liver						
Absolute	1.502 ± 0.046	1.390 ± 0.041	1.430 ± 0.045	1.542 ± 0.060	1.448 ± 0.044	1.527 ± 0.052
Relative	40.132 ± 1.334	38.456 ± 1.113	40.501 ± 0.891	41.059 ± 1.492	43.058 ± 1.381	46.073 ± 1.213**
Lung						
Absolute	0.180 ± 0.006	0.180 ± 0.004	0.185 ± 0.011	0.183 ± 0.003	0.175 ± 0.002	0.186 ± 0.006
Relative	4.806 ± 0.172	4.977 ± 0.095	5.301 ± 0.427	4.875 ± 0.082	5.204 ± 0.086	5.671 ± 0.322*
R. Testis						
Absolute	0.114 ± 0.004	0.124 ± 0.001*	0.117 ± 0.002	0.121 ± 0.003	0.119 ± 0.002	0.119 ± 0.003
Relative	3.046 ± 0.118	3.441 ± 0.063	3.330 ± 0.066	3.227 ± 0.066	3.544 ± 0.094**	3.632 ± 0.190**
Thymus						
Absolute	0.036 ± 0.004	0.032 ± 0.002	0.035 ± 0.002	0.036 ± 0.002	0.032 ± 0.001	0.033 ± 0.002
Relative	0.941 ± 0.080	0.889 ± 0.049	0.983 ± 0.045	0.957 ± 0.050	0.940 ± 0.040	0.990 ± 0.065
Female						
Necropsy body wt	29.6 ± 0.8	29.6 ± 0.8	31.7 ± 1.0	29.3 ± 0.6	30.0 ± 0.8	27.8 ± 0.7
Heart						
Absolute	0.137 ± 0.004	0.137 ± 0.003	0.135 ± 0.006	0.139 ± 0.005	0.141 ± 0.005	0.132 ± 0.003
Relative	4.642 ± 0.148	4.645 ± 0.116	4.299 ± 0.247	4.770 ± 0.224	4.741 ± 0.221	4.769 ± 0.141
R. Kidney						
Absolute	0.216 ± 0.007	0.213 ± 0.008	0.223 ± 0.006	0.225 ± 0.009	0.218 ± 0.007	0.214 ± 0.006
Relative	7.342 ± 0.301	7.233 ± 0.309	7.070 ± 0.239	7.719 ± 0.367	7.323 ± 0.331	7.704 ± 0.129
Liver						
Absolute	1.299 ± 0.044	1.345 ± 0.037	1.401 ± 0.045	1.345 ± 0.026	1.378 ± 0.026	1.400 ± 0.040
Relative	44.022 ± 1.551	45.552 ± 1.029	44.482 ± 1.910	46.097 ± 1.321	46.226 ± 1.439	50.439 ± 1.248**
Lung						
Absolute	0.195 ± 0.012	0.181 ± 0.007	0.191 ± 0.010	0.195 ± 0.006	0.186 ± 0.008	0.179 ± 0.005
Relative	6.584 ± 0.338	6.139 ± 0.238	6.074 ± 0.389	6.665 ± 0.205	6.278 ± 0.397	6.456 ± 0.194
Thymus						
Absolute	0.044 ± 0.002	0.047 ± 0.002	0.042 ± 0.002	0.043 ± 0.002	0.040 ± 0.002	0.040 ± 0.002
Relative	1.482 ± 0.050	1.595 ± 0.080	1.345 ± 0.080	1.458 ± 0.076	1.327 ± 0.067	1.438 ± 0.072

* 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).

TABLE G6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 12-Month Interim Evaluation in the 2-Year Feed Study of Emodin[a]

	0 ppm	160 ppm	312 ppm	625 ppm
n	10	10	10	10
Male				
Necropsy body wt	48.0 ± 1.0	49.6 ± 1.3	47.5 ± 1.2	47.6 ± 1.1
R. Kidney				
Absolute	0.402 ± 0.013	0.399 ± 0.015	0.394 ± 0.009	0.403 ± 0.014
Relative	8.383 ± 0.218	8.038 ± 0.189	8.331 ± 0.216	8.452 ± 0.175
L. Kidney				
Absolute	0.379 ± 0.018	0.381 ± 0.015	0.371 ± 0.011	0.379 ± 0.010
Relative	7.877 ± 0.285	7.669 ± 0.179	7.827 ± 0.162	7.959 ± 0.136
Liver				
Absolute	2.056 ± 0.103	2.082 ± 0.100	1.831 ± 0.081	1.906 ± 0.100
Relative	42.836 ± 1.945	41.909 ± 1.540	38.480 ± 1.048	39.827 ± 1.323
	0 ppm	**312 ppm**	**625 ppm**	**1,250 ppm**
Female				
Necropsy body wt	50.7 ± 2.1	51.0 ± 1.8	46.7 ± 3.0	48.4 ± 2.0
R. Kidney				
Absolute	0.252 ± 0.005	0.236 ± 0.010	0.240 ± 0.008	0.229 ± 0.007
Relative	5.018 ± 0.149	4.639 ± 0.150	5.390 ± 0.529	4.779 ± 0.170
L. Kidney				
Absolute	0.239 ± 0.006	0.220 ± 0.011	0.227 ± 0.007	0.206 ± 0.007*
Relative	4.759 ± 0.154	4.320 ± 0.172	5.113 ± 0.501	4.309 ± 0.190
Liver				
Absolute	1.572 ± 0.058	1.516 ± 0.070	1.523 ± 0.092	1.469 ± 0.075
Relative	31.131 ± 0.752	29.705 ± 0.800	33.328 ± 1.957	30.345 ± 0.713

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

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1 Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Feed Study of Emodin 232
TABLE H2 Summary of Estrous Cycle Characterization for Female Rats in the 14-Week Feed Study of Emodin 232
TABLE H3 Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Feed Study of Emodin 233
TABLE H4 Summary of Estrous Cycle Characterization for Female Mice in the 14-Week Feed Study of Emodin 233

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Feed Study of Emodin[a]

	0 ppm	312.5 ppm	1,250 ppm	5,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	361 ± 5	363 ± 4	364 ± 7	325 ± 6**
L. cauda epididymis	0.2134 ± 0.0044	0.2170 ± 0.0036	0.2160 ± 0.0030	0.2158 ± 0.0030
L. epididymis	0.4677 ± 0.0078	0.4852 ± 0.0046	0.4746 ± 0.0058	0.4828 ± 0.0067
L. testis	1.5504 ± 0.0208	1.5506 ± 0.0195	1.5627 ± 0.0257	1.5432 ± 0.0234
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.33 ± 0.63	10.09 ± 0.67	8.93 ± 0.47	9.20 ± 0.80
Spermatid heads (10^7/testis)	14.47 ± 0.98	15.69 ± 1.12	13.92 ± 0.70	14.23 ± 1.30
Spermatid count (mean/10^{-4} mL suspension)	72.33 ± 4.91	78.43 ± 5.62	69.60 ± 3.52	71.13 ± 6.50
Epididymal spermatozoal measurements				
Motility (%)	90.48 ± 0.78	91.17 ± 0.58	90.48 ± 0.30	91.84 ± 0.70
Concentration (10^6/g cauda epididymal tissue)	546 ± 39	621 ± 29	609 ± 32	590 ± 28

** Significantly different (P≤0.01) from the control group by William'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
Summary of Estrous Cycle Characterization for Female Rats in the 14-Week Feed Study of Emodin[a]

	0 ppm	312.5 ppm	1,250 ppm	5,000 ppm
n	10	10	10	10
Necropsy body wt (g)	196 ± 2	198 ± 4	188 ± 2*	178 ± 2**
Estrous cycle length (days)	4.50 ± 0.15	4.95 ± 0.16	5.10 ± 0.22*	5.60 ± 0.19**
Estrous stages (% of cycle)				
Diestrus	34.2	35.0	35.0	42.5
Proestrus	14.2	14.2	14.2	14.2
Estrus	29.2	29.2	30.8	26.7
Metestrus	22.5	21.7	20.0	16.7

* Significantly different (P≤0.05) from the control group by William's test (body weight) or Shirley's test (estrous cycle length)
** P≤0.01
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. 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.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Feed Study of Emodin[a]

	0 ppm	312.5 ppm	1,250 ppm	5,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	37.6 ± 1.0	36.2 ± 0.5	37.6 ± 0.6	33.3 ± 1.2**
L. cauda epididymis	0.0212 ± 0.0015	0.0211 ± 0.0009	0.0189 ± 0.0010	0.0176 ± 0.0005
L. epididymis	0.0482 ± 0.0029	0.0497 ± 0.0010	0.0472 ± 0.0014	0.0458 ± 0.0008
L. testis	0.1080 ± 0.0044	0.1199 ± 0.0019*	0.1173 ± 0.0027	0.1171 ± 0.0027
Spermatid measurements				
Spermatid heads (10^7/g testis)	21.33 ± 1.18	17.96 ± 1.28	21.33 ± 0.92	18.56 ± 0.82
Spermatid heads (10^7/testis)	2.30 ± 0.17	2.14 ± 0.14	2.50 ± 0.11	2.17 ± 0.11
Spermatid count (mean/10^{-4} mL suspension)	72.03 ± 5.26	66.95 ± 4.31	77.98 ± 3.50	67.90 ± 3.58
Epididymal spermatozoal measurements				
Motility (%)	91.41 ± 2.48	93.38 ± 0.42	93.71 ± 0.44	94.42 ± 0.50
Concentration (10^6/g cauda epididymal tissue)	1,256 ± 67	1,199 ± 70	1,339 ± 91	1,387 ± 53

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

TABLE H4
Summary of Estrous Cycle Characterization for Female Mice in the 14-Week Feed Study of Emodin[a]

	0 ppm	312.5 ppm	1,250 ppm	5,000 ppm
n	10	10	10	10
Necropsy body wt (g)	29.6 ± 0.8	29.6 ± 0.8	29.3 ± 0.6	27.8 ± 0.7
Estrous cycle length (days)	4.05 ± 0.05	4.10 ± 0.07	4.10 ± 0.10	4.10 ± 0.07
Estrous stages (% of cycle)				
Diestrus	30.8	30.8	29.2	34.2
Proestrus	21.7	23.3	20.0	22.5
Estrus	27.5	26.7	30.8	25.8
Metestrus	20.0	18.3	20.0	16.7
Uncertain diagnoses	0.0	0.8	0.0	0.8

[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body weight) or 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.

APPENDIX I
DETERMINATIONS OF EMODIN IN PLASMA

TABLE I1 Plasma Concentrations of Emodin in Rats at the 3-Month Interim Evaluation in the 2-Year Feed Study of Emodin 236
TABLE I2 Plasma Concentrations of Emodin in Rats at the 6-Month Interim Evaluation in the 2-Year Feed Study of Emodin 236
TABLE I3 Plasma Concentrations of Emodin in Rats at the 12-Month Interim Evaluation in the 2-Year Feed Study of Emodin 237

TABLE I1
Plasma Concentrations of Emodin in Rats at the 3-Month Interim Evaluation in the 2-Year Feed Study of Emodin[a]

	280 ppm	830 ppm	2,500 ppm
n	3	3	3
Male			
Time of collection			
0800	0.058 ± 0.001[b]	0.367 ± 0.001[b]	1.505 ± 0.105[b]
1700	0.053 ± 0.015	0.179 ± 0.005	1.012 ± 0.228
2000	0.048 ± 0.009[b]	0.208 ± 0.020[b]	1.285 ± 0.165[b]
0300	0.082 ± 0.015	0.274 ± 0.030	1.407 ± 0.143
Female			
Time of collection			
0800	0.189 ± 0.025[b]	0.443 ± 0.056[b]	1.350 ± 0.190[b]
1700	0.045 ± 0.005	0.210 ± 0.024	0.630 ± 0.028
2000	0.101 ± 0.006[b]	0.339 ± 0.089[b]	0.822 ± 0.124[b]
0300	0.163 ± 0.014	0.346 ± 0.044	1.115 ± 0.212

[a] Data are given in μg/mL as mean ± standard error.
[b] n=2

TABLE I2
Plasma Concentrations of Emodin in Rats at the 6-Month Interim Evaluation in the 2-Year Feed Study of Emodin[a]

	280 ppm	830 ppm	2,500 ppm
n	3	3	3
Male			
Time of collection			
0800	0.070 ± 0.021[b]	0.373 ± 0.016[b]	0.794 ± 0.019[b]
1700	0.046 ± 0.006	0.088 ± 0.008	0.272 ± 0.078
2000	0.053 ± 0.013[b]	0.343 ± 0.074[b]	0.821 ± 0.055[b]
0300	0.097 ± 0.004	0.436 ± 0.030	0.681 ± 0.092
Female			
Time of collection			
0800	0.118 ± 0.012[b]	0.439 ± 0.057[b]	0.724 ± 0.135[b]
1700	0.087 ± 0.015	0.303 ± 0.021	0.584 ± 0.088
2000	0.111 ± 0.011[b]	0.467 ± 0.062[b]	1.000 ± 0.051[b]
0300	0.113 ± 0.017	0.594 ± 0.138	1.141 ± 0.336

[a] Data are given in μg/mL as mean ± standard error.
[b] n=2

TABLE I3
Plasma Concentrations of Emodin in Rats at the 12-Month Interim Evaluation in the 2-Year Feed Study of Emodin[a]

	280 ppm	830 ppm	2,500 ppm
n	2	3	3
Male			
Time of collection			
0800	0.090 ± 0.023	0.213 ± 0.037[c]	0.406 ± 0.055[c]
1700	0.026 ± 0.008	0.116 ± 0.042	0.222 ± 0.009
2000	0.033 ± 0.003	0.180 ± 0.018[c]	0.418 ± 0.108[c]
0300	0.058 ± 0.021[b]	0.220 ± 0.057	0.491 ± 0.007
Female			
Time of collection			
0800	0.062 ± 0.010	0.221 ± 0.081[c]	0.965 ± 0.125[c]
1700	0.021 ± 0.007	0.217 ± 0.028	0.759 ± 0.142
2000	0.023 ± 0.005	0.210 ± 0.010[c]	0.755 ± 0.010[c]
0300	0.053[d]	0.205 ± 0.014	—[e]

[a] Data are given in μg/mL as mean ± standard error.
[b] n=3
[c] n=2
[d] n=1; no standard error calculated
[e] Due to an interfering peak, the emodin concentration could not be accurately measured.

APPENDIX J
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF EMODIN 240
PREPARATION AND ANALYSIS OF DOSE FORMULATIONS 241
FIGURE J1 Infrared Absorption Spectrum of Emodin 243
FIGURE J2 Nuclear Magnetic Resonance Spectrum of Emodin 244
TABLE J1 High-Performance Liquid Chromatography Systems Used in the Feed Studies of Emodin 245
TABLE J2 Preparation and Storage of Dose Formulations in the Feed Studies of Emodin 246
TABLE J3 Results of Analyses of Dose Formulations Administered to Rats and Mice in the 16-Day Feed Studies of Emodin 247
TABLE J4 Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of Emodin 248
TABLE J5 Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Emodin 249
TABLE J6 Results of Referee Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of Emodin 256

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF EMODIN

Emodin was obtained from Aldrich Chemical Company (Milwaukee, WI) in four lots (99912ET, EW11728DW, 06625DG, and 13309EG) and from Pfaltz & Bauer, Inc. (Waterbury, CT), in one lot (040221). Lot 99912ET was used during the 16-day studies, and lot EW11728DW was used during the 14-week studies. All lots were combined as described below as lot SRI-A09/L7 and used during the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) and the study laboratory; stability analyses were conducted by the analytical chemistry laboratory. Reports on analyses performed in support of the emodin studies are on file at the National Institute of Environmental Health Sciences.

To prepare lot SRI-A09/L7, lots 99912ET, EW11728DW, and 040221 were blended as lot SRI-A09/L5; lots 06625DG and 13309EG were blended as lot SRI-A09/L6. These two mixtures were then blended together.

The chemical, a fluffy, orange powder, was identified as emodin by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with the literature references (Bachmann and Schlatter, 1981; *Merck Index*, 1989; *Sadtler Standard Spectra*) of emodin. The infrared and nuclear magnetic resonance spectra are presented in Figures J1 and J2. The melting point ranges of 253.9° to 255.4° C (lot 99912ET), 253.7° to 255.9° C (lot EW11728DW), and 255.0° to 256.5° C (lot SRI-A09/L7) were consistent with a range of 256° to 257° C cited in the literature (*Merck Index*, 1989).

The purity of lots 99912ET and EW11728DW was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC) at the analytical chemistry laboratory. The purity of lot SRI-A09/L7 was determined by elemental analyses, Karl Fischer water analysis, gas chromatography/mass spectroscopy (GC/MS), and HPLC at the study laboratory. For functional group titration of phenols, emodin samples were dissolved in 2-propanol and titrated with 0.1 N tetrabutylammonium hydroxide. All titrations were monitored potentiometrically with a glass indicating electrode and a calomel reference electrode filled with methanolic 1 M tetrabutylammonium chloride. TLC was performed on Silica Gel 60 F-254 plates with two solvent systems: 1) toluene:chloroform:acetone (35:25:40) and 2) toluene:acetone (95:5). Phenol in ethanol was used as a reference standard. The plates were examined with four different visualization methods: visible light, ultraviolet light at 254 and 366 nm, and a spray with two different reagents. For the spray, the first reagent was a 0.4% methanolic solution of 2,6-dichloroquinonechloroimide, and the second reagent was 10% aqueous sodium carbonate; plates were sprayed with each reagent and then dried at 120° C for 3 to 5 minutes. HPLC by systems similar to system A (Table J1) were used to characterize the emodin used in the 16-day, 14-week, and 2-year studies. HPLC by system A (lot 99912ET), system B (lot EW11728DW), or system C (lot SRI-A09/L7) was used to measure impurities in each lot. GC/MS analysis of lot SRI-A09/L7 was performed with a Model 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a Model 5971A mass selective detector (Hewlett-Packard). The temperature program was 45° C for 3 minutes, then 45° to 300° C at 8° C per minute.

For lot 99912ET, elemental analyses were in agreement with the theoretical values for emodin. Karl Fischer water analysis indicated less than 0.4% water. Functional group titration indicated a purity of 101.1% ± 0.6%. TLC by the first system indicated a major peak and three trace impurities; TLC by the second system indicated a major peak and two trace impurities. HPLC at each wavelength (254 and

436 nm) indicated one major peak and four impurities with combined areas of 1.8% and 1.5%, respectively, relative to the major peak area. The cumulative data indicated a purity of approximately 98%.

For lot EW11728DW, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for emodin. Karl Fischer water analysis indicated 0.34% ± 0.03% water. Functional group titration indicated a purity of 93.7% ± 0.5%. TLC by both systems revealed one major peak and two minor impurities. HPLC at both wavelengths indicated one major peak and three impurities with combined areas of 7.0% and 6.2%, respectively, relative to the major peak area. Major peak comparisons of lot EW11728DW with lot 99912ET by HPLC indicated a purity of 96.4% ± 0.5% for lot EW11728DW relative to lot 99912ET. The overall purity of lot EW11728DW was determined to be approximately 94%. Lot EW11728DW was further characterized by HPLC/MS (system B). Two impurity peaks with areas greater than 1% relative to the major peak were resolved by this system; one impurity, with a molecular weight of 254, was tentatively identified as 1,8-dihydroxy-3-methylanthraquinone, and the other, with a molecular weight of 284, as 1,8-dihydroxy-3-methoxy-6-methylanthraquinone. HPLC using conditions similar to those in system A and with decanophenone as an internal standard was used to confirm the identity of and quantitate the impurity with the molecular weight of 254; no standard for the second impurity was commercially available. The presence of 2.13% ± 0.2% 1,8-dihydroxy-3-methylanthraquinone was confirmed.

For lot SRI-A09/L7, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for emodin. Karl Fischer water analysis indicated 0.94% ± 0.13% water. GC/MS indicated one major peak and two impurities with a combined area of 3.4% relative to the major peak area; mass spectra of these impurities were identical to those of chrysophenic acid and physcion. HPLC indicated one major peak and two impurities with a combined area of 2.94% relative to the major peak area. The retention times confirmed the identities of the impurities as chrysophanic acid and physcion. The overall purity of lot SRI-A09/L7 was approximately 96.1%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using HPLC (similar to system A). These studies indicated that emodin is stable for at least 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature in sealed containers (16-day studies: amber glass bottles; 14-week and 2-year studies: doubled plastic bags) protected from light. Stability was monitored throughout the studies using HPLC by system C (16-day and 14-week studies) or system A (2-year studies). No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once for the 16-day studies, and every 2 weeks for the 14-week and 2-year studies by mixing emodin with feed (Table J2). A premix of emodin and feed was prepared by hand and then blended with feed in a Patterson-Kelly twin-shell blender for 15 minutes using an intensifier bar for the initial 5 minutes. Formulations were stored in plastic bags in opaque plastic buckets at room temperature for up to 3 weeks.

Homogeneity and stability studies of a 500 ppm dose formulation were performed by the analytical chemistry laboratory using HPLC (system C). Homogeneity was confirmed, and the stability of the dose formulations was confirmed for 3 weeks at 5° C when stored in the dark. Dose formulations stored open to air and light lost 4.4% emodin after 4 days and 6.3% after 7 days. Homogeneity studies of the 600 and 50,000 ppm dose formulations for the 16-day studies, all concentrations used in the 14-week studies, and the 160 ppm and 2,500 ppm dose formulations for the 2-year studies were performed by the study

laboratory using HPLC with conditions similar to system C (16-day and 2-year studies) or system A (14-week studies).

Periodic analyses of the dose formulations of emodin were conducted at the study laboratory using HPLC (similar to system C) for all studies. Dose formulations were analyzed at the beginning of the 16-day studies (Table J3). Doses were analyzed at the beginning, midpoint, and end of the 14-week studies (Table J4). During the 2-year studies, formulations were analyzed approximately every 10 weeks (Table J5). All five dose formulations in the 16-day studies were within 10% of the target concentrations. Of the dose formulations analyzed during the 14-week studies, 20 of 22 were within 10% of the target concentrations. The out-of-range dose formulations were remixed and found to be within acceptable limits. Of the dose formulations analyzed during the 2-year studies, 148 of 156 for rats and 63 of 64 for mice were within 10% of the target concentration with no value greater than 113% (rats) or 114% (mice) of the target concentration. One out-of-range dose formulation for rats and one for mice were remixed. The remaining out-of-range rat dose formulations were used; this did not affect the study results. Results of periodic referee analyses performed by the analytical chemistry laboratory during the 14-week studies agreed with the results obtained by the study laboratory (Table J6).

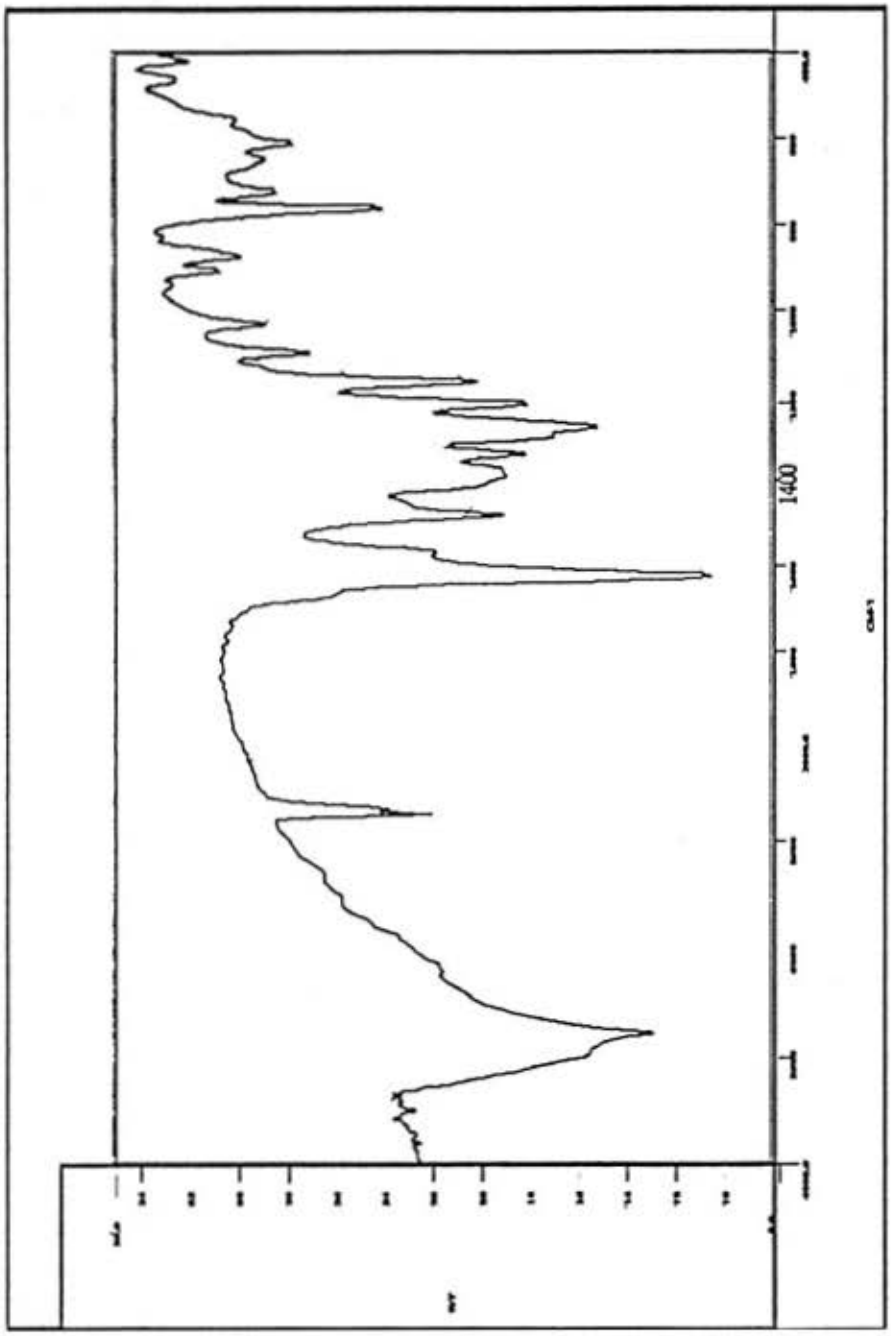


FIGURE J1
Infrared Absorption Spectrum of Emodin

Assignments (δ ppm)	Multiplicity	Integration Observed	Integration Theoretical
(a) 2.42	s	2.8	3
(b) 6.52	d	1.3	1
	$J_{b\text{-}d}$ = ~ 2.4 Hz		
(c) 7.06	s	1.6	2
(d) 7.14	d		
(e) 7.52	s	1.2	1

Solvent and Internal Reference
TMS 0.00
Methanol 3.31, 4.87

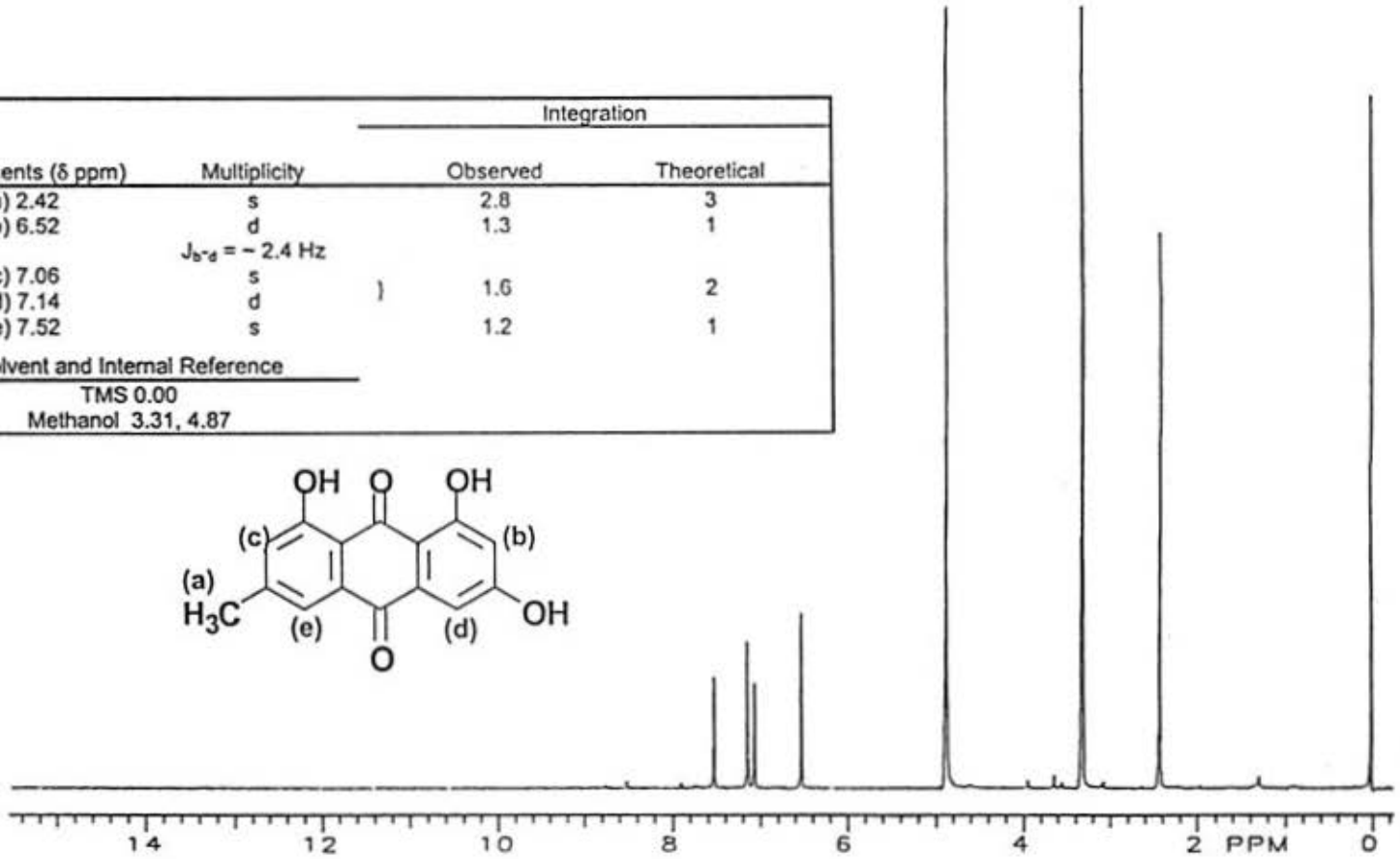


FIGURE J2
Nuclear Magnetic Resonance Spectrum of Emodin

TABLE J1
High-Performance Liquid Chromatography Systems Used in the Feed Studies of Emodin[a]

Detection System	Column	Solvent System
System A Ultraviolet (254 nm) and visible (436 nm) light	Waters Resolve C_{18}, 150 mm × 3.9 mm, 5 μm (Waters-Millipore, Milford, MA)	A) Water with 1% glacial acetic acid and B) methanol with 1% glacial acetic acid (35% A:65% B), isocratic; flow rate 1.0 mL/minute
System B Ultraviolet (210 nm) light coupled with mass spectrometry	Perkin Elmer C_{18}, 30 mm × 4.6 mm, 3 μm (Perkin Elmer, Norwalk, CT)	A) Water with 1% glacial acetic acid and B) methanol with 1% glacial acetic acid; linear gradient from 30% A:70% B to 15% A:85% B in 5 minutes at a flow rate of 1.0 mL/minute
System C Ultraviolet (254 nm) light	Waters μBondapak C_{18}, 300 mm × 3.9 mm, 10 μm	Water:ethanol:acetic acid (32:68:1); flow rate 1.0 mL/minute; octanophenone as internal standard

[a] High-performance liquid chromatographs were manufactured by Waters-Millipore (Milford, MA).

TABLE J2
Preparation and Storage of Dose Formulations in the Feed Studies of Emodin

16-Day Studies	14-Week Studies	2-Year Studies
Preparation		
A premix of feed and emodin was prepared, then layered with 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. Dose formulations were prepared at the beginning of the studies.	Same as 16-day studies. Dose formulations were prepared every 2 weeks.	Same as 16-day studies. Dose formulations were prepared every 2 weeks.
Chemical Lot Number		
99912ET	EW11728DW	SRI-A09/L7
Maximum Storage Time		
3 weeks	3 weeks	3 weeks
Storage Conditions		
Stored in double-thickness opaque plastic bags inside opaque plastic buckets, protected from light, at room temperature	Same as 16-day studies	Stored in double amber or opaque plastic bags inside opaque plastic buckets at approximately 5° C
Study Laboratory		
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
Referee Laboratory		
None	Midwest Research Institute (Kansas City, MO)	None

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 16-Day Feed Studies of Emodin

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration[a] (mg/g)	Difference from Target (%)
15 April 1988	18-20 April 1988[b]	0.6	0.626	+4
		0.6	0.652	+9
		0.6	0.627	+5
		50	52.9	+6
		50	52.2	+4
		50	52.0	+4
	18-20 April 1988	0.6	0.638	+6
		2.0	2.02	+1
		5.5	5.50	0
		17	17.4	+2
		50	51.4	+3

[a] Results of duplicate analyses. 0.6 mg/g=600 ppm; 2.0 mg/g=2,000 ppm; 5.5 mg/g=5,500 ppm; 17 mg/g=17,000 ppm; and 50 mg/g=50,000 ppm

[b] Homogeneity analyses of formulations used for dosing. For each target concentration, the three results given are the concentrations determined from samples collected from the top right, top left, and bottom of the twin-shell blender.

TABLE J4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of Emodin

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration[a] (mg/g)	Difference from Target (%)
20 October 1989	20-24 October 1989	0.3125	0.327	+5
		0.3125	0.324	+4
		0.625	0.622	0
		0.625	0.622	0
		1.25	1.18	−6
		1.25	1.22	−2
		2.5	2.34	−6
		2.5	2.34	−6
		5	4.70	−6
		5	4.88	−2
1 December 1989	4-5 December 1989	0.3125	0.352	+13
		0.625	0.668	+7
		1.25	1.26	+1
		2.5	2.62	+5
		5	5.88	+18
5 December 1989	5-6 December 1989	0.3125	0.320[b]	+2
		5	5.04[b]	+1
26 January 1990	29-30 January 1990	0.3125	0.302	−3
		0.625	0.627	0
		1.25	1.16	−7
		2.5	2.41	−4
		5	4.97	−1

[a] Results of duplicate analyses. 0.3125 mg/g=312.5 ppm; 0.625 mg/g=625 ppm; 1.25 mg/g=1,250 ppm; 2.5 mg/g=2,500 ppm; and 5 mg/g=5,000 ppm
[b] Results of remix

TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Emodin

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration[a] (mg/g)	Difference from Target (%)
Rats				
23-24 August 1994	24-26 August 1994	0.28	0.269	−4
		0.28	0.266	−5
		0.28	0.263	−6
		0.28	0.271	−3
		0.83	0.85	+2
		0.83	0.852	+3
		0.83	0.842	+1
		0.83	0.89	+7
		2.5	2.66	+6
		2.5	2.72	+9
		2.5	2.72	+9
		2.5	2.71	+8
	14-15 September 1994[b]	0.28	0.201	−28
		0.28	0.242	−14
		0.28	0.279	0
		0.83	0.665	−20
		0.83	0.736	−11
		0.83	0.733	−12
		0.83	0.708	−15
		2.5	2.1	−16
		2.5	2.34	−6
		2.5	2.34	−6
		2.5	2.23	−11
31 October - 1 November 1994	1-3 November 1994	0.28	0.268	−4
		0.28	0.261	−7
		0.28	0.266	−5
		0.28	0.267	−5
		0.83	0.755	−9
		0.83	0.761	−8
		0.83	0.779	−6
		0.83	0.803	−3
		2.5	2.41	−4
		2.5	2.38	−5
		2.5	2.39	−4
		2.5	2.41	−4
12-13 December 1994	19-21 December 1994	0.28	0.26	−7
		0.28	0.278	−1
		0.28	0.278	−1
		0.28	0.281	0
		0.83	0.828	0
		0.83	0.807	−3
		0.83	0.818	−1
		0.83	0.825	−1
		2.5	2.55	+2
		2.5	2.44	−2
		2.5	2.46	−2
		2.5	2.76	+10
20-21 February 1995	22-25 February 1995	0.28	0.262	−6
		0.28	0.27	−4

TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Emodin

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
20-21 February 1995	22-25 February 1995	0.28	0.268	–4
		0.28	0.267	–5
		0.28	0.265	–5
		0.83	0.837	+1
		0.83	0.869	+5
		0.83	0.86	+4
		0.83	0.833	0
		0.83	0.834	0
		2.5	2.53	+1
		2.5	2.52	+1
		2.5	2.54	+2
		2.5	2.52	+1
		2.5	2.51	0
	27 March - 4 April 1995[b]	0.28	0.403[c]	+44
		0.28	0.484[c]	+73
		0.28	0.456[c]	+63
		0.28	0.288	+3
		0.83	0.705[c]	–15
		0.83	0.754	–9
		0.83	0.716	–14
		0.83	0.827	0
		2.5	2.45	–2
		2.5	2.51[c]	0
		2.5	2.54	+2
	7 April 1995[b]	0.28	0.359	+28
		0.28	0.414	+48
		0.28	0.394	+41
		0.83	0.703	–15
		2.5	2.46	–2
	14-15 April 1995[b]	0.28	0.244	–13
		0.28	0.271	–3
		0.28	0.311	+11
		0.83	0.818	–1
		2.5	2.56	+2
1-2 May 1995	1-4 May 1995	0.28	0.264	–6
		0.28	0.286	+2
		0.28	0.257	–8
		0.28	0.266	–5
		0.28	0.266	–5
		0.83	0.757	–9
		0.83	0.741	–11[d]
		0.83	0.806	–3
		0.83	0.815	–2
		0.83	0.817	–2
		2.5	2.44	–2
		2.5	2.46	–2
		2.5	2.26	–10
		2.5	2.4	–4
		2.5	2.44	–2

TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Emodin

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
10 July 1995	10-12 July 1995	0.28	0.311	+11[d]
		0.28	0.307	+10
		0.28	0.311	+11[d]
		0.28	0.311	+11[d]
		0.28	0.311	+11[d]
		0.83	0.853	+3
		0.83	0.855	+3
		0.83	0.851	+3
		0.83	0.863	+4
		0.83	0.844	+2
		2.5	2.68	+7
		2.5	2.67	+7
		2.5	2.67	+7
		2.5	2.69	+8
		2.5	2.7	+8
19 September 1995	19-22 September 1995	0.28	0.289	+3
		0.28	0.296	+6
		0.28	0.29	+4
		0.28	0.277	−1
		0.28	0.271	−3
		0.83	0.858	+3
		0.83	0.796	−4
		0.83	0.783	−6
		0.83	0.817	−2
		0.83	0.94	+13[d]
		2.5	2.64	+6
		2.5	2.71	+8
		2.5	2.82	+13[d]
		2.5	2.58	+3
		2.5	2.54	+2
	9-11 October 1995[b]	0.28	0.239	−15
		0.28	0.245	−12
		0.28	0.244	−13
		0.83	0.685	−17
		0.83	0.684	−18
		0.83	0.76	−8
		0.83	0.756	−9
		0.83	0.754	−9
		2.5	2.57	+3
		2.5	2.54	+2
		2.5	2.62	+5
		2.5	2.46	−2
		2.5	2.47	−1
	19 October 1995[b]	0.28	0.304[e]	+9
		0.28	0.226[e]	−19

TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Emodin

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
27 November 1995	28-29 November 1995	0.28	0.302	+8
		0.28	0.299	+7
		0.28	0.287	+3
		0.28	0.286	+2
		0.28	0.297	+6
		0.83	0.827	0
		0.83	0.757	–9
		0.83	0.76	–8
		0.83	0.752	–9
		0.83	0.778	–6
		2.5	2.49	0
		2.5	2.25	–10
		2.5	2.42	–3
		2.5	2.42	–3
		2.5	2.44	–2
21 February 1996	22-23 February 1996	0.28	0.279	0
		0.28	0.296	+6
		0.28	0.274	–2
		0.28	0.295	+5
		0.28	0.298	+6
		0.83	0.765	–8
		0.83	0.833	0
		0.83	0.831	0
		0.83	0.813	–2
		0.83	0.733	–12
		2.5	2.62	+5
		2.5	2.6	+4
		2.5	2.6	+4
		2.5	2.61	+4
		2.5	2.65	+6
28 February 1996	29 February 1996	0.83	0.835[f]	+1
29-30 April 1996	30 April - 2 May 1996	0.28	0.286	+2
		0.28	0.289	+3
		0.28	0.277	–1
		0.28	0.274	–2
		0.28	0.286	+2
		0.83	0.801	–3
		0.83	0.79	–5
		0.83	0.785	–5
		0.83	0.834	0
		0.83	0.837	+1
		2.5	2.53	+1
		2.5	2.49	0
		2.5	2.51	0
		2.5	2.48	–1
		2.5	2.44	–2
	21 May 1996[b]	0.28	0.245	–12
		0.83	0.772	–7
		2.5	2.29	–8

TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Emodin

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Rats (continued)				
9-10 July 1996	9-13 July 1996	0.28	0.284	+1
		0.28	0.294	+5
		0.28	0.295	+5
		0.28	0.285	+2
		0.28	0.298	+6
		0.83	0.819	−1
		0.83	0.814	−2
		0.83	0.785	−5
		0.83	0.829	0
		0.83	0.787	−5
		2.5	2.42	−3
		2.5	2.26	−10
		2.5	2.35	−6
		2.5	2.31	−8
		2.5	2.26	−10
Mice				
23-24 August 1994	24-26 August 1994	0.160	0.172	+8
		0.312	0.284	−9
		0.625	0.611	−2
		1.25	1.29	+3
	14-15 September 1994[b]	0.160	0.16	0
		0.312	0.29	−7
		0.625	0.596	−5
		1.25	1.15	−8
31 October - 1 November 1994	1-3 November 1994	0.160	0.156	−2
		0.312	0.295	−5
		0.312	0.288	−8
		0.625	0.581	−7
		0.625	0.577	−8
		1.25	1.23	−2
12-13 December 1994	19-21 December 1994	0.160	0.165	+3
		0.312	0.318	+2
		0.312	0.302	−3
		0.625	0.621	−1
		0.625	0.609	−3
		1.25	1.2	−4

TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Emodin

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice (continued)				
20-21 February 1995	22-25 February 1995	0.160	0.158	−1
		0.312	0.298	−4
		0.312	0.305	−2
		0.625	0.617	−1
		0.625	0.64	+2
		1.25	1.23	−2
	27 March - 4 April 1995[b]	0.160	0.281[c]	+76
		0.312	0.28	−10
		0.625	0.515	−18
		1.25	1.23	−2
	7 April 1995[b]	0.16	0.247	+54
	14-15 April 1995[b]	0.16	0.174	+9
1-2 May 1995	1-4 May 1995	0.160	0.162	+1
		0.312	0.292	−6
		0.312	0.293	−6
		0.625	0.568	−9
		0.625	0.567	−9
		1.25	1.2	−4
10 July 1995	10-12 July 1995	0.160	0.166	+4
		0.312	0.338	+8
		0.312	0.324	+4
		0.625	0.633	+1
		0.625	0.641	+3
		1.25	1.3	+4
19 September 1995	19-22 September 1995	0.160	0.171	+7
		0.312	0.321	+3
		0.312	0.299	−4
		0.625	0.581	−7
		0.625	0.619	−1
		1.25	1.27	+2
	9-11 October 1995[b]	0.160	0.161	+1
		0.312	0.243	−22
		0.312	0.224	−28
		0.625	0.451	−28
		0.625	0.449	−28
		1.25	1.17	−6
27 November 1995	28-29 November 1995	0.160	0.182	+14
		0.312	0.296	−5
		0.312	0.331	+6
		0.625	0.606	−3
		0.625	0.618	−1
		1.25	1.21	−3
6 December 1995	6-7 December 1995	0.160	0.169[f]	+6
		0.160	0.16[f]	0
		0.160	0.161[f]	+1

TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Emodin

Date Prepared	Date Analyzed	Target Concentration (mg/g)	Determined Concentration (mg/g)	Difference from Target (%)
Mice (continued)				
21 February 1996	22-23 February 1996	0.160	0.152	−5
		0.312	0.297	−5
		0.312	0.321	+3
		0.625	0.631	+1
		0.625	0.567	−9
		1.25	1.23	−2
29-30 April 1996	30 April - 2 May 1996	0.160	0.165	+3
		0.312	0.315	+1
		0.312	0.31	−1
		0.625	0.614	−2
		0.625	0.599	−4
		1.25	1.23	−2
	21 May 1996[b]	0.160	0.13	−19
		0.312	0.241	−23
		0.625	0.523	−16
		1.25	1.1	−12
9-10 July 1996	9-13 July 1996	0.160	0.172	+8
		0.312	0.306	−2
		0.312	0.343	+10
		0.625	0.599	−4
		0.625	0.607	−3
		1.25	1.13	−10

[a] Results of duplicate analyses. 0.160 mg/g=160 ppm; 0.28 mg/g=280 ppm; 0.312 mg/g=312 ppm; 0.625 mg/g=625 ppm; 0.83 mg/g=830 ppm; 1.25 mg/g=1,250 ppm; and 2.5 mg/g=2,500 ppm
[b] Animal room samples
[c] Due to low E/O (expected/observed) ratios of animal room samples analyzed on 27 March - 4 April 1995, samples were analyzed in triplicate on 7 April 1995. Additional aliquots of these samples were analyzed in duplicate on 14-15 April 1995.
[d] Formulation mistakenly used in study
[e] Results of triplicate analyses; reanalysis results for animal room samples with low E/O ratios determined on 19-22 September 1995
[f] Results of remix

TABLE J6
Results of Referee Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of Emodin

Date Prepared	Target Concentration (mg/g)	Determined Concentration (mg/g)	
		Study Laboratory[a]	Referee Laboratory[b]
20 October 1989	0.625	0.622	0.609 ± 0.008
1 December 1989	2.5	2.62	2.47 ± 0.03

[a] Results of duplicate analyses. 0.625 mg/g=625 ppm and 2.5 mg/g=2,500 ppm
[b] Results of triplicate analyses (mean ± standard error)

APPENDIX K
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES OF EMODIN

TABLE K1 **Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Emodin** 258
TABLE K2 **Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Emodin** 259
TABLE K3 **Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of Emodin** 260
TABLE K4 **Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Emodin** 261

TABLE K1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Emodin

	0 ppm		280 ppm			830 ppm			2,500 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
2	16.3	178	16.0	175	256	15.8	171	766	14.4	160	2,245
5	18.0	264	18.5	260	200	17.4	253	573	17.4	236	1,842
9	17.1	325	17.3	319	152	16.6	310	443	16.0	291	1,373
13	16.5	359	16.6	356	130	16.0	344	387	15.9	330	1,206
17	16.2	386	16.0	382	118	15.7	373	349	15.6	356	1,097
21	16.6	405	16.8	400	117	16.2	390	345	15.9	369	1,080
25	16.1	420	16.0	416	108	15.7	404	324	15.8	387	1,019
29	16.8	438	16.9	431	110	16.8	423	330	16.5	398	1,037
33	17.7	448	17.9	443	113	17.4	434	333	17.4	412	1,052
37	15.7	461	15.5	457	95	15.4	445	288	14.9	427	875
41	16.0	464	16.0	457	98	15.7	449	290	15.3	431	890
45	16.7	467	16.1	465	97	16.0	458	291	15.8	436	905
49	15.3	471	15.3	468	91	14.8	460	268	14.7	440	833
53	15.2	473	14.7	469	88	14.7	458	266	15.0	441	850
57	15.0	474	16.2	472	96	16.2	464	291	15.8	444	888
61	15.1	472	15.1	468	90	15.5	459	281	14.8	440	843
65	14.9	471	14.8	465	89	15.2	458	275	15.2	440	863
69	15.2	472	15.0	465	90	15.7	459	283	15.4	440	876
73	15.0	471	15.2	464	92	15.4	457	280	15.4	443	867
77	15.2	471	14.6	464	88	14.8	457	268	14.2	442	806
81	15.5	472	14.8	462	89	14.9	454	273	15.2	443	857
85	15.1	465	14.9	460	91	15.9	456	289	15.9	443	899
89	15.2	459	14.2	449	89	14.2	444	265	14.4	434	831
93	14.5	456	14.4	445	91	13.2	431	255	14.2	430	823
97	13.4	452	13.2	432	85	13.5	429	261	13.8	427	805
101	14.2	449	13.9	427	91	13.5	420	267	12.9	417	775
Mean for weeks											
1-13	16.9	282	17.1	277	185	16.5	269	542	15.9	254	1,666
14-52	16.3	440	16.3	435	105	16.0	426	313	15.8	406	976
53-101	14.9	466	14.7	457	90	14.8	450	273	14.8	437	845

[a] Grams of feed consumed per animal per day
[b] Milligrams of emodin consumed per kilogram body weight per day

TABLE K2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Emodin

	0 ppm		280 ppm			830 ppm			2,500 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
2	11.8	131	11.7	129	254	11.6	128	753	11.2	124	2,260
5	11.6	163	12.0	161	209	11.4	157	603	11.0	152	1,816
9	10.4	183	10.3	180	160	10.1	177	473	9.9	173	1,428
13	10.0	195	9.8	192	143	9.3	189	411	9.8	186	1,320
17	9.7	205	9.7	203	134	9.9	199	415	9.9	198	1,247
21	10.0	211	9.8	209	132	9.7	205	390	9.2	202	1,136
25	9.7	219	9.4	214	124	9.4	211	368	9.8	209	1,166
29	10.0	226	10.1	222	127	9.8	217	373	10.6	217	1,227
33	10.6	235	10.6	228	129	10.4	226	380	10.3	222	1,157
37	9.7	241	9.6	233	115	9.5	230	344	9.5	224	1,055
41	10.3	247	9.7	240	114	9.9	237	348	10.1	232	1,091
45	10.1	255	10.2	247	115	10.1	243	345	10.0	237	1,055
49	10.5	265	9.9	253	110	10.0	250	331	9.8	245	1,002
53	10.3	268	10.1	256	111	10.1	253	330	10.0	247	1,013
57	10.8	274	10.7	263	114	10.9	260	348	11.0	253	1,081
61	10.9	282	10.9	269	114	10.7	268	332	10.9	260	1,045
65	11.1	292	10.5	277	106	11.0	278	328	10.9	272	1,001
69	11.3	301	11.5	286	112	11.3	287	326	11.6	279	1,039
73	11.5	308	11.1	293	106	11.3	294	319	11.5	289	991
77	11.5	319	11.0	302	102	11.2	300	311	11.2	296	946
81	11.3	324	10.1	303	94	11.5	308	310	10.7	296	906
85	12.0	331	12.3	316	109	11.5	314	304	11.8	304	971
89	11.8	333	11.6	317	102	11.9	318	311	11.1	302	922
93	10.8	330	11.6	319	102	11.5	317	300	11.7	308	949
97	11.6	336	11.5	326	99	11.2	320	291	11.1	310	896
101	11.2	336	11.9	331	101	12.0	331	300	11.4	314	907
Mean for weeks											
1-13	10.9	168	10.9	166	191	10.6	163	560	10.5	159	1,706
14-52	10.1	234	9.9	228	122	9.8	224	366	9.9	221	1,126
53-101	11.2	310	11.1	297	106	11.2	296	316	11.1	287	974

[a] Grams of feed consumed per animal per day
[b] Milligrams of emodin consumed per kilogram body weight per day

TABLE K3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of Emodin

	0 ppm		160 ppm			312 ppm			625 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
1	4.3	23.8	4.3	23.8	29	4.4	24.0	57	4.3	23.5	114
2	5.5	25.7	6.1	25.5	38	5.5	25.7	67	5.9	25.5	146
4	4.5	27.8	4.5	27.4	26	4.8	27.6	54	4.8	27.5	109
8	4.5	31.4	4.6	31.2	24	4.6	31.3	46	4.7	31.2	94
12	4.3	34.3	4.3	33.8	20	4.4	34.0	41	4.4	33.9	82
17	4.4	37.7	4.6	37.3	20	4.5	37.5	38	4.6	37.8	76
21	4.5	40.5	4.6	40.1	19	4.7	40.5	37	4.7	40.3	73
25	4.5	41.7	4.4	41.2	17	4.5	41.5	34	4.5	41.4	68
29	4.4	44.6	4.3	43.4	16	4.3	43.5	31	4.4	43.4	63
33	4.4	46.2	4.5	44.7	16	4.6	45.3	32	4.6	45.0	64
37	4.4	48.0	4.3	47.0	15	4.4	47.2	29	4.3	46.8	57
41	4.3	48.4	4.3	47.8	14	4.2	47.9	27	4.3	47.3	57
45	4.3	48.9	4.3	48.5	14	4.4	48.5	28	4.4	48.2	57
49	4.4	49.5	4.4	49.0	14	4.4	49.0	28	4.5	48.8	58
53	4.6	49.2	4.6	48.8	15	4.6	48.5	30	4.5	48.2	59
57	4.8	49.9	4.7	48.7	15	4.8	49.1	30	4.7	48.6	61
61	5.0	50.2	4.9	49.2	16	5.1	50.0	32	5.1	49.3	65
65	4.8	50.4	4.8	49.3	16	5.0	50.0	31	4.9	49.4	63
69	4.8	50.5	4.9	49.7	16	5.0	50.0	31	4.9	49.8	62
73	4.8	51.1	4.8	50.2	15	4.9	50.1	31	4.9	49.8	61
77	4.9	50.2	4.9	49.1	16	5.1	49.2	32	4.9	49.0	63
81	4.7	50.0	5.0	48.7	16	5.0	49.3	32	4.8	49.0	62
85	4.4	50.3	4.4	49.3	14	4.6	50.6	29	4.6	49.6	59
89	4.4	49.9	4.3	48.6	14	4.6	49.8	29	4.6	49.2	58
93	4.4	50.6	4.5	49.7	14	4.4	50.0	28	4.3	49.4	54
97	4.6	50.1	4.5	49.2	15	4.5	49.5	29	4.4	48.7	57
101	4.5	49.6	4.4	48.7	15	4.5	48.8	29	4.5	48.3	58
Mean for weeks											
1-13	4.6	28.6	4.8	28.3	28	4.7	28.5	53	4.8	28.3	109
14-52	4.4	45.1	4.4	44.3	16	4.5	44.5	32	4.5	44.3	64
53-101	4.7	50.2	4.7	49.2	15	4.8	49.6	30	4.7	49.1	60

[a] Grams of feed consumed per animal per day
[b] Milligrams of emodin consumed per kilogram body weight per day

TABLE K4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Emodin

	0 ppm		312 ppm			625 ppm			1,250 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
1	3.5	18.5	3.3	18.5	56	3.4	18.5	116	3.2	18.6	214
2	3.6	20.7	3.7	20.4	57	3.6	20.5	108	3.5	20.3	214
4	3.3	19.6	3.8	21.4	56	3.6	20.7	108	3.8	21.7	220
8	3.7	23.0	3.6	23.3	48	3.6	23.2	96	3.7	24.1	194
12	3.9	26.3	3.8	26.4	45	3.8	26.4	90	3.8	26.9	177
16	3.9	29.9	3.8	29.9	39	4.0	30.6	81	3.9	30.6	160
20	4.0	31.6	4.1	32.6	39	4.2	33.2	79	4.0	32.6	155
24	3.8	34.5	3.9	34.9	35	3.9	35.1	70	4.1	36.0	141
28	4.1	36.8	3.8	36.9	32	3.9	37.4	66	3.7	37.4	125
32	4.1	39.4	4.0	39.0	32	4.1	39.8	64	4.1	39.2	130
36	3.9	41.6	3.8	41.5	29	3.9	42.2	57	4.2	41.8	125
40	4.2	44.7	4.0	43.6	28	3.9	44.6	55	4.0	43.6	114
44	3.8	45.5	3.9	45.3	27	3.9	46.5	53	3.8	45.2	105
48	3.6	46.2	3.5	46.3	23	3.5	46.7	47	3.2	45.7	88
52	4.0	48.5	3.9	47.2	26	3.9	48.1	51	3.9	47.8	103
56	4.5	49.8	4.5	48.6	29	4.6	50.3	57	4.5	49.3	113
60	4.5	50.7	4.4	50.9	27	4.3	51.4	52	4.1	51.0	101
64	4.2	51.6	4.4	52.3	26	4.5	52.5	54	4.2	52.0	101
68	4.3	53.3	4.5	54.3	26	4.5	54.7	51	4.4	53.8	103
72	4.5	56.1	4.7	55.7	26	4.5	55.9	50	4.5	54.8	102
76	4.1	55.6	4.4	56.0	24	4.4	55.7	49	4.2	55.2	96
80	4.4	56.5	4.7	56.3	26	4.4	56.3	49	4.5	55.6	100
84	4.1	58.0	4.2	57.5	23	4.1	57.3	45	4.0	56.5	88
88	4.1	58.7	4.3	57.5	23	4.2	58.2	45	3.9	56.2	86
92	4.1	57.2	4.4	57.1	24	4.4	57.3	48	4.3	55.5	97
96	4.6	58.5	4.6	58.1	25	4.5	58.1	48	4.5	55.3	101
100	4.2	57.7	4.1	57.0	22	4.1	56.0	46	4.2	54.2	97
Mean for weeks											
1-13	3.6	21.6	3.7	22.0	53	3.6	21.9	104	3.6	22.3	204
14-52	3.9	39.9	3.9	39.7	31	3.9	40.4	62	3.9	40.0	125
53-100	4.3	55.3	4.4	55.1	25	4.4	55.3	50	4.3	54.1	99

[a] Grams of feed consumed per animal per day
[b] Milligrams of emodin consumed per kilogram body weight per day

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

TABLE L1 Ingredients of NIH-07 Rat and Mouse Ration 264
TABLE L2 Vitamins and Minerals in NIH-07 Rat and Mouse Ration 264
TABLE L3 Nutrient Composition of NIH-07 Rat and Mouse Ration 265
TABLE L4 Contaminant Levels in NIH-07 Rat and Mouse Ration 266

TABLE L1
Ingredients of NIH-07 Rat and Mouse Ration[a]

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

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

TABLE L2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE L3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.94 ± 0.55	22.1 | 24.2	24
Crude fat (% by weight)	5.25 ± 0.26	4.60 | 5.70	24
Crude fiber (% by weight)	3.44 ± 0.27	2.80 | 4.00	24
Ash (% by weight)	6.42 ± 0.22	6.05 | 7.06	24
Amino Acids (% of total diet)			
Arginine	1.272 ± 0.083	1.100 | 1.390	12
Cystine	0.307 ± 0.068	0.181 | 0.400	12
Glycine	1.152 ± 0.051	1.060 | 1.220	12
Histidine	0.581 ± 0.029	0.531 | 0.630	12
Isoleucine	0.913 ± 0.034	0.867 | 0.965	12
Leucine	1.969 ± 0.053	1.850 | 2.040	12
Lysine	1.269 ± 0.050	1.200 | 1.370	12
Methionine	0.436 ± 0.104	0.306 | 0.699	12
Phenylalanine	0.999 ± 0.114	0.665 | 1.110	12
Threonine	0.899 ± 0.059	0.824 | 0.985	12
Tryptophan	0.216 ± 0.146	0.107 | 0.671	12
Tyrosine	0.690 ± 0.091	0.564 | 0.794	12
Valine	1.079 ± 0.057	0.962 | 1.170	12
Essential Fatty Acids (% of total diet)			
Linoleic	2.389 ± 0.223	1.830 | 2.570	11
Linolenic	0.273 ± 0.034	0.210 | 0.320	11
Vitamins			
Vitamin A (IU/kg)	6,623 ± 403	4,780 | 7,480	24
Vitamin D (IU/kg)	4,450 ± 1,382	3,000 | 6,300	4
α-Tocopherol (ppm)	35.24 ± 8.58	22.5 | 48.9	12
Thiamine (ppm)	18.60 ± 3.79	13.3 | 26.0	24
Riboflavin (ppm)	7.78 ± 0.899	6.10 | 9.00	12
Niacin (ppm)	98.73 ± 23.21	65.0 | 150.0	12
Pantothenic acid (ppm)	32.94 ± 8.92	23.0 | 59.2	12
Pyridoxine (ppm)	9.28 ± 2.49	5.60 | 14.0	12
Folic acid (ppm)	2.56 ± 0.70	1.80 | 3.70	12
Biotin (ppm)	0.265 ± 0.046	0.190 | 0.354	12
Vitamin B_{12} (ppb)	41.6 ± 18.6	10.6 | 65.0	12
Choline (ppm)	2,955 ± 382	2,300 | 3,430	11
Minerals			
Calcium (%)	1.18 ± 0.08	1.06 | 1.36	24
Phosphorus (%)	0.94 ± 0.05	0.85 | 1.10	24
Potassium (%)	0.886 ± 0.059	0.772 | 0.971	10
Chloride (%)	0.531 ± 0.082	0.380 | 0.635	10
Sodium (%)	0.316 ± 0.031	0.258 | 0.370	12
Magnesium (%)	0.165 ± 0.010	0.148 | 0.180	12
Sulfur (%)	0.266 ± 0.060	0.208 | 0.420	11
Iron (ppm)	348.0 ± 83.7	255.0 | 523.0	12
Manganese (ppm)	93.27 ± 5.62	81.7 | 102.0	12
Zinc (ppm)	59.42 ± 9.73	46.1 | 81.6	12
Copper (ppm)	11.63 ± 2.46	8.09 | 15.4	12
Iodine (ppm)	3.49 ± 1.14	1.52 | 5.83	11
Chromium (ppm)	1.57 ± 0.53	0.60 | 2.09	12
Cobalt (ppm)	0.81 ± 0.27	0.49 | 1.23	8

TABLE L4
Contaminant Levels in NIH-07 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.52 ± 0.19	0.10 | 0.80	24
Cadmium (ppm)	0.06 ± 0.03	0.04 | 0.13	24
Lead (ppm)	0.23 ± 0.09	0.11 | 0.50	24
Mercury (ppm)	<0.02		24
Selenium (ppm)	0.31 ± 0.10	0.10 | 0.50	24
Aflatoxins (ppb)	<5.0		24
Nitrate nitrogen (ppm)[c]	7.96 ± 4.21	0.80 | 16.5	24
Nitrite nitrogen (ppm)[c]	1.14 ± 1.18	0.04 | 4.80	24
BHA (ppm)[d]	1.09 ± 1.10	0.01 | 5.00	24
BHT (ppm)[d]	1.43 ± 1.15	0.10 | 5.00	24
Aerobic plate count (CFU/g)	308,250 ± 344,356	43,000 | 1,200,000	24
Coliform (MPN/g)	582 ± 1,103	4 | 4,300	24
Escherichia coli (MPN/g)	<10		24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[e]	10.83 ± 4.00	2.9 | 19.7	24
N-Nitrosodimethylamine (ppb)[e]	9.06 ± 3.91	1.9 | 18.00	24
N-Nitrosopyrrolidine (ppb)[e]	1.77 ± 0.74	1.0 | 3.9	24
Pesticides (ppm)			
α-BHC	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.10		24
Estimated PCBs	<0.20		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.10		24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion	0.13 ± 0.18	0.03 | 0.91	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

[a] CFU=colony forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] 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 M
SENTINEL ANIMAL PROGRAM

METHODS 268
RESULTS 270

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 randomly selected rats and mice during the 14-week and 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 Microbiological Associates, Inc. (Bethesda, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
14-Week Study	
ELISA	
PVM (pneumonia virus of mice)	Study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	Study termination
KRV (Kilham rat virus)	Study termination
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	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
Hemagglutination Inhibition	
H-1	6, 12, and 18 months, study termination
KRV	6, 12, and 18 months, study termination

Method and Test	Time of Analysis
MICE	
14-Week Study	
ELISA	
Ectromelia virus	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
Mouse adenoma virus	Study termination
MHV (mouse hepatitis virus)	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Immunofluorescence Assay	
EDIM (epizootic diarrhea of infant mice)	Study termination
MVM (minute virus of mice)	Study termination
Hemagglutination Inhibition	
K (papovavirus)	Study termination
Polyoma virus	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
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	
EDIM	18 months
GDVII	12 months and study termination
LCM	Study termination
Mouse adenoma virus-FL	Study termination
MCMV (mouse cytomegalovirus)	Study termination
MHV	18 months and study termination
Reovirus 3	Study termination
Hemagglutination Inhibition	
K	6, 12, and 18 months, study termination
MVM	6, 12, and 18 months, study termination
Polyoma virus	6, 12, and 18 months, study termination

RESULTS

One rat and one mouse had positive titers for *M. arthritidis* at the end of the 2-year studies. Further evaluation of samples positive for *M. arthritidis* by immunoblot and Western blot procedures indicated that the positive titers may have been due to cross reaction with antibodies of nonpathogenic *Mycoplasma* or other agents. Only two samples were positive and there were no clinical findings or histopathologic changes of *M. arthritidis* infection in animals with positive titers. Accordingly, *M. arthritidis*-positive titers were considered to be false positives.

APPENDIX N
SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F$_1$ MICE

RESULTS 272
TABLE N1 Plasma Concentrations of Emodin in F344/N Rats after a Single Intravenous Injection of 5 mg/kg Emodin 273
FIGURE N1 Plasma Concentrations of Emodin in F344/N Rats after a Single Intravenous Injection of 5 mg/kg Emodin 274
TABLE N2 Summary of Toxicokinetic Data from a Single-Dose Intravenous Injection Study of Emodin in F344/N Rats 275
TABLE N3 Plasma Concentrations of Emodin in F344/N Rats after a Single Gavage Dose of Emodin 275
TABLE N4 Plasma Concentrations of Emodin in B6C3F$_1$ Mice after a Single Intravenous Injection of 10 mg/kg Emodin 276
FIGURE N2 Plasma Concentrations of Emodin in B6C3F$_1$ Mice after a Single Intravenous Injection of 10 mg/kg Emodin 277
TABLE N5 Summary of Toxicokinetic Data from a Single-Dose Intravenous Injection Study of Emodin in B6C3F$_1$ Mice 278
TABLE N6 Plasma Concentrations of Emodin in B6C3F$_1$ Mice after a Single Gavage Dose of Emodin 278

SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F$_1$ MICE

RESULTS

Rats

Intravenous Administration

Following the single intravenous dose administration of 5 mg/kg, male plasma concentrations decreased from a mean of 9.2 μg/mL at 2 minutes to below the limit of quantitation (0.10 μg/mL) at 90 minutes (Table N1 and Figure N1); female plasma concentrations decreased from a mean of 5.82 μg/mL at 2 minutes to below the limit of quantitation at 120 minutes.

Observed toxicokinetic parameters included a maximum emodin plasma concentration (C_{max}) of 9.15 μg/mL (males) or 5.82 μg/mL (females) at 540 minutes after dosing (T_{max}) (Table N2). The $t_{½}$ was determined to be 0.174 hours for males and 0.28 hours for females and biphasic for both males and females. The AUC was 3.50 μg/mL●min for males and 1.49 μg/mL●min for females.

Oral Gavage Administration

No measurable emodin concentrations were found in the plasma of male or female rats after a single gavage administration of 20 or 40 mg/kg emodin (Table N3); trace amounts (less than 0.12 μg/mL) at 360 and 480 minutes occurred in 40 mg/kg females. In 80 mg/kg males, a C_{max} (mean 0.145 μg/mL) was observed at 540 minutes post-administration; low concentrations were observed in males at 360 and 720 minutes. In 80 mg/kg females, a C_{max} (mean 0.233 μg/mL) was observed at 540 minutes, and small concentrations of emodin were observed at 180 to 720 minutes after dosing. Areas under the concentration versus time curve (AUCs) were much less than would be expected for intravenous AUCs, indicating incomplete absorption, strong first-pass hepatic metabolism, or a combination of the two.

Mice

Intravenous Administration

Following the single intravenous dose administration of 10 mg/kg, plasma concentrations decreased from a mean of 7.06 μg/mL (males) or 3.83 μg/mL (females) at 2 minutes to below the limit of quantitation (0.10 μg/mL) at 120 minutes (Table N4 and Figure N2).

Observed toxicokinetic parameters included a maximum emodin plasma concentration (C_{max}) of 7.06 μg/mL (males) or 3.83 μg/mL (females) at 540 minutes after dosing (T_{max}) (Table N5). The $t_{½}$ was determined to be 0.38 hours and biphasic for females; the $t_{½}$ for males was not determined. The AUC was 1.62 μg/mL●min for males and 1.13 μg/mL●min for females.

Oral Gavage Administration

No measurable emodin concentrations were found in the plasma of male mice after a single gavage administration of 20 mg/kg emodin (Table N6); trace amounts (less than 0.135 μg/mL) at 60 minutes occurred in 20 mg/kg females. Less than 0.175 μg/mL of emodin was observed at 40 and 180 minutes in 40 mg/kg males; less than 0.236 μg/mL emodin was observed at 40, 90, and 180 minutes in 40 mg/kg females. In 80 mg/kg males and females, less than 0.55 μg/mL emodin was found at 40, 90, and 180 minutes. Areas under the concentration versus time curve (AUCs) were much less than would be expected for intravenous AUCs, indicating incomplete absorption, strong first-pass hepatic metabolism, or a combination of the two.

TABLE N1
Plasma Concentrations of Emodin in F344/N Rats after a Single Intravenous Injection of 5 mg/kg Emodin[a]

Time after Dosing (minutes)	Concentration[b] (μg/mL)
Male	
2	9.2 ± 1.2
5	8.23 ± 0.36
10	6.07 ± 1.36
20	5.09 ± 0.79
40	1.96 ± 0.25
60	0.218 ± 0.012
90	<LOQ
120	<LOQ
180	<LOQ
240	<LOQ
Female	
2	5.82 ± 0.77
5	4.33 ± 1.13
10	2.31 ± 0.68
20	1.46 ± 0.84
40	0.877 ± 0.120
60	0.192 ± 0.055
90	0.133 ± 0.017
120	<LOQ
180	<LOQ
240	<LOQ

[a] Three animals were bled at each time point. LOQ=limit of quantitation (0.10 μg/mL)
[b] Mean ± standard deviation

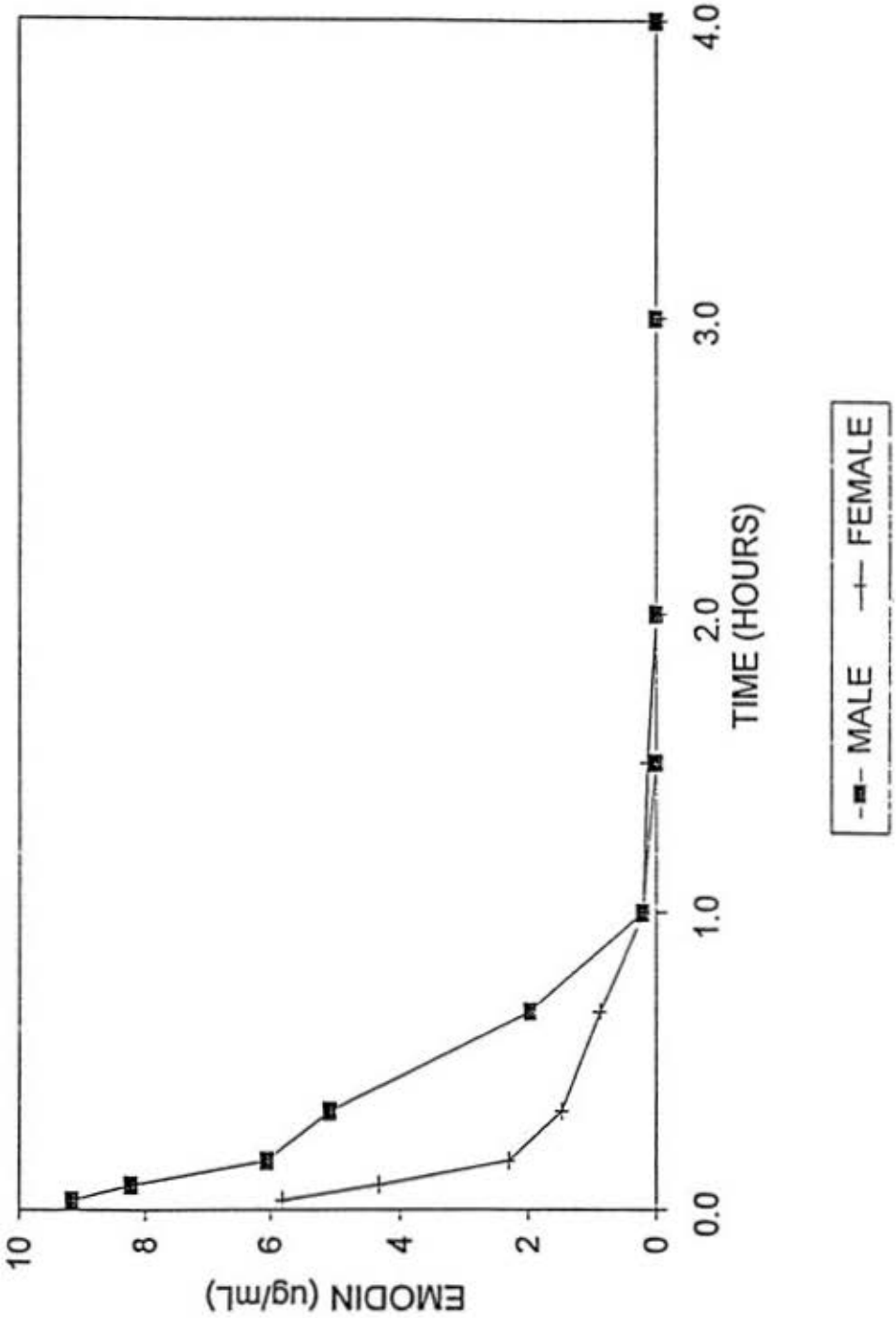


FIGURE N1
Plasma Concentrations of Emodin in F344/N Rats after a Single Intravenous Injection of 5 mg/kg Emodin

TABLE N2
Summary of Toxicokinetic Data from a Single-Dose Intravenous Injection Study of Emodin in F344/N Rats[a]

Route	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (hours)	$t_{½}$ (hours)	AUC (μg•hr/mL)
Male					
Intravenous injection	5	9.15	0.033	0.174	3.50
Female					
Intravenous injection	5	5.82	0.033	0.28	1.49

[a] C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration; $t_{½}$=elimination half-life; AUC=area under the curve

TABLE N3
Plasma Concentrations of Emodin in F344/N Rats after a Single Gavage Dose of Emodin[a]

20 mg/kg		40 mg/kg		80 mg/kg	
Time after Dosing (minutes)	Concentration[b] (μg/mL)	Time after Dosing (minutes)	Concentration (μg/mL)	Time after Dosing (minutes)	Concentration (μg/mL)
Male					
10	<LOQ	10	<LOQ	10	<LOQ
30	<LOQ	40	<LOQ	40	<LOQ
60	<LOQ	90	<LOQ	90	<LOQ
120	<LOQ	180	<LOQ	180	<LOQ
240	<LOQ	360	<LOQ	360	0.134 ± 0.036[c]
360	<LOQ	480	<LOQ	540	0.145 ± 0.016
540	<LOQ	660	<LOQ	720	0.113 ± 0.004[c]
720	<LOQ	840	<LOQ	960	<LOQ
Female					
10	<LOQ	10	<LOQ	10	<LOQ
30	<LOQ	40	<LOQ	40	<LOQ
60	<LOQ	90	<LOQ	90	<LOQ
120	<LOQ	180	<LOQ	180	0.169 ± 0.076
240	<LOQ	360	<LOQ	360	0.153 ± 0.011
360	<LOQ	480	<LOQ	540	0.233 ± 0.036
540	<LOQ	660	<LOQ	720	0.175 ± 0.009
720	<LOQ	840	<LOQ	960	<LOQ

[a] Three animals were bled at each time point. LOQ=limit of quantitation (0.10 μg/mL)
[b] Mean ± standard deviation
[c] n=2; one of the three samples was below the LOQ.

TABLE N4
Plasma Concentrations of Emodin in B6C3F$_1$ Mice after a Single Intravenous Injection of 10 mg/kg Emodin[a]

Time after Dosing (minutes)	Concentration[b] (μg/mL)
Male	
2	7.06 ± 1.26
5	4.49 ± 0.46
10	3.32 ± 0.67
20	0.573 ± 0.145
40	0.330 ± 0.118
60	1.04 ± 1.01
90	0.122 ± 0.011[c]
120	<LOQ
180	<LOQ
240	<LOQ
Female	
2	3.83 ± 0.03
5	2.50 ± 0.11
10	2.89 ± 1.19
20	0.646 ± 0.146
40	0.448 ± 0.176
60	0.198 ± 0.063
90	0.138 ± 0.036
120	<LOQ
180	<LOQ
240	<LOQ

[a] Three animals were bled at each time point. LOQ=limit of quantitation (0.10 μg/mL)
[b] Mean ± standard deviation
[c] n=2; third sample was below the LOQ.

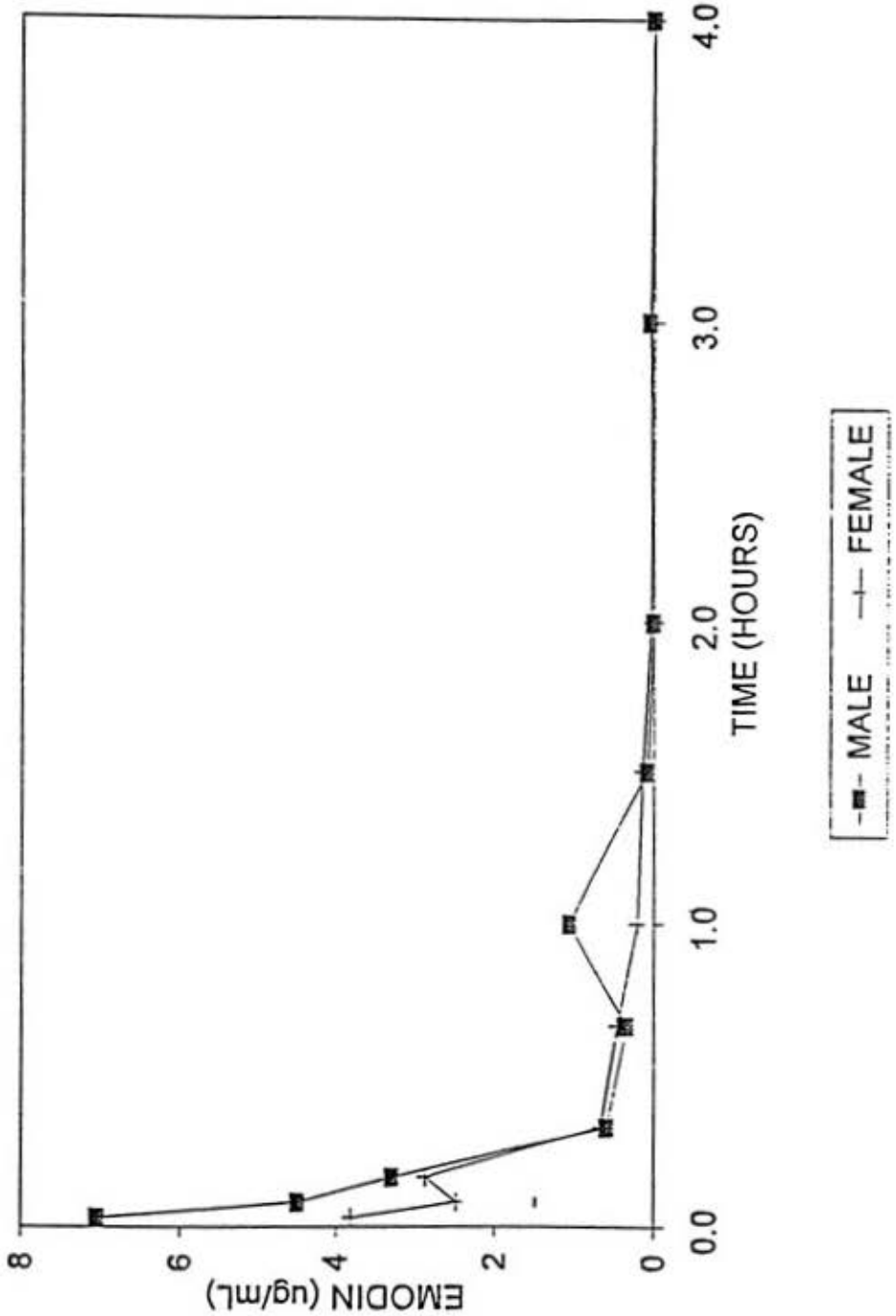


FIGURE N2
Plasma Concentrations of Emodin in B6C3F$_1$ Mice after a Single Intravenous Injection of 10 mg/kg Emodin

TABLE N5
Summary of Toxicokinetic Data from a Single-Dose Intravenous Injection Study of Emodin in B6C3F$_1$ Mice[a]

Route	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (hours)	$t_{½}$ (hours)	AUC (μg•hr/mL)
Male					
Intravenous injection	10	7.06	0.033	—[b]	1.62
Female					
Intravenous injection	10	3.83	0.033	0.383	1.13

[a] C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration; $t_{½}$=elimination half-life; AUC=area under the curve
[b] Not determined

TABLE N6
Plasma Concentrations of Emodin in B6C3F$_1$ Mice after a Single Gavage Dose of Emodin[a]

20 mg/kg		40 mg/kg		80 mg/kg	
Time after Dosing (minutes)	Concentration[b] (μg/mL)	Time after Dosing (minutes)	Concentration (μg/mL)	Time after Dosing (minutes)	Concentration (μg/mL)
Male					
10	<LOQ	10	<LOQ	10	<LOQ
30	<LOQ	40	0.131 ± 0.030	40	0.367 ± 0.112
60	<LOQ	90	<LOQ	90	0.152 ± 0.059
120	<LOQ	180	0.150 ± 0.019	180	0.157 ± 0.023
240	<LOQ	360	<LOQ	360	<LOQ
360	<LOQ	480	<LOQ	540	<LOQ
540	<LOQ	660	<LOQ	720	<LOQ
720	<LOQ	840	<LOQ	960	<LOQ
Female					
10	<LOQ	10	<LOQ	10	<LOQ
30	<LOQ	40	0.186 ± 0.045	40	0.305 ± 0.119
60	0.126 ± 0.013[c]	90	0.155 ± 0.044[c]	90	0.297 ± 0.191
120	<LOQ	180	0.168 ± 0.014[c]	180	0.252 ± 0.248
240	<LOQ	360	<LOQ	360	<LOQ
360	<LOQ	480	<LOQ	540	<LOQ
540	<LOQ	660	<LOQ	720	<LOQ
720	<LOQ	840	<LOQ	960	<LOQ

[a] Three animals were bled at each time point. LOQ=limit of quantitation (0.10 mg/mL)
[b] Mean ± standard deviation
[c] n=2; one of the three samples was below the LOQ.