NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF ANTHRAQUINONE

(CAS NO. 84-65-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

Scheduled Peer Review Date: February 18, 2004

NOTICE

This is a DRAFT Technical Report prepared for public review and comment. Until this DRAFT has been reviewed and approved by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee in public session, the interpretations described herein do not represent the official scientific position of the National Toxicology Program. Following peer review, readers should contact the NTP for the final version of this Technical Report.

NTP TR 494

NIH Publication No. 04-3953

National Toxicology Program

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 Perspectives (EHP) http://ehp.niehs.nih.gov (866-541-3841 or 919-653-2590). In addition, printed copies of these reports are available from EHP as supplies last.

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CONTENTS

ABSTRACT 5

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY 14

TECHNICAL REPORTS REVIEW SUBCOMMITTEE 15

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS 17

INTRODUCTION 21

MATERIALS AND METHODS 31

RESULTS 49

DISCUSSION AND CONCLUSIONS 105

REFERENCES 123

APPENDIX A Summary of Lesions in Male Rats in the 2-Year Feed Study of Anthraquinone A-1

APPENDIX B Summary of Lesions in Female Rats in the 2-Year Feed Study of Anthraquinone B-1

APPENDIX C Summary of Lesions in Male Mice in the 2-Year Feed Study of Anthraquinone C-1

APPENDIX D Summary of Lesions in Female Mice in the 2-Year Feed Study of Anthraquinone D-1

APPENDIX E Genetic Toxicology E-1

APPENDIX F Clinical Pathology Results F-1

APPENDIX G Organ Weights and Organ-Weight-to-Body-Weight Ratios G-1

APPENDIX H Reproductive Tissue Evaluations and Estrous Cycle Characterization H-1

APPENDIX I Pharmacokinetic Model and Toxicokinetic Results I-1

APPENDIX J Chemical Characterization and Dose Formulation Studies J-1

APPENDIX K Feed and Compound Consumption in the 2-Year Feed Studies of Anthraquinone . . K-1

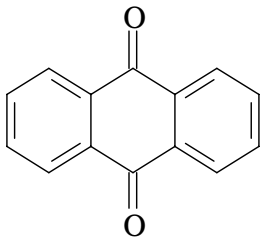
APPENDIX L Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration L-1

APPENDIX M Sentinel Animal Program M-1

APPENDIX N Single-Dose Toxicokinetic Studies in F344/N Rats and B6C3F$_1$ Mice N-1

APPENDIX O 32-Day Feed Study of Anthraquinone in F344/N Rats O-1

ABSTRACT



ANTHRAQUINONE

CAS No. 84-65-1

Chemical Formula: $C_{14}H_8O_2$ Molecular Weight: 208.22

Synonyms: 9,10-Anthracenedione; anthradione; 9,10-anthraquinone; 9,10-dioxoanthracene; 9,10-dihydro-9,10-dioxoanthracene
Trade names: Corbit, Hoelite, Morkit

Anthraquinone is used as an intermediate in the manufacture of dyes and pigments, an additive in the kraft pulping process in the paper industry, a catalyst in the isomerization of vegetable oils, an accelerator in nickel electroplating, and as a bird repellant. The National Toxicology Program is conducting a class study of naturally occurring quinones containing the anthraquinone ring; anthraquinone is the parent compound of this class. Male and female F344/N rats and B6C3F$_1$ mice were exposed to anthraquinone (approximately **99.9% pure by gas chromatography**) in feed for 14 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse bone marrow cells, and mouse peripheral blood erythrocytes.

14-Week Study in Rats

Groups of 10 male and 10 female F344/N rats were fed diets containing 0, 1,875, 3,750, 7,500, 15,000, or 30,000 ppm anthraquinone (equivalent to average daily doses of approximately 135, 275, 555, 1,130, or 2,350 mg anthraquinone/kg body weight) for 14 weeks. All rats survived until the end of the study. Mean body weights of exposed groups of females were significantly less than that of the control group. Feed

consumption by exposed groups was similar to that by the control groups at the end of the study. Liver and kidney weights of exposed groups were greater than those of the controls, as were testis weights of males exposed to 7,500 ppm or greater. A minimal, responsive anemia was apparent in groups of male and female rats exposed to 3,750 ppm or greater by day 26 of the study. The anemia persisted and involved all exposed groups of rats at the end of the study. Renal function was also affected by anthraquinone exposure as demonstrated by increases in urine protein and glucose concentrations and aspartate aminotransferase and *N*-acetyl-β-D-glucosaminidase activities. Estrous cycles of 15,000 and 30,000 ppm females were longer than that of the controls.

Groups of exposed rats had liver hypertrophy; eosinophilic hyaline droplets in the kidney; congestion, hematopoietic cell proliferation, and pigmentation of the spleen; and bone marrow hyperplasia. The incidences of nephropathy in 15,000 and 30,000 ppm females were significantly greater than that in the controls, and the severities of nephropathy were increased in exposed groups of males and in 30,000 ppm females. The concentrations of α2u-globulin in the kidneys were significantly greater in all exposed groups of males. Thyroid gland follicular cell hypertrophy was present in all males and females exposed to 3,750 ppm or greater. Incidences of inflammation and transitional cell hyperplasia in the urinary bladder of 30,000 ppm females were greater than those in the controls.

14-Week Study in Mice

Groups of 10 male and 10 female B6C3F$_1$ mice were fed diets containing 0, 1,875, 3,750, 7,500, 15,000, or 30,000 ppm anthraquinone (equivalent to average daily doses of approximately 250, 500, 1,050, 2,150, or 4,300 mg/kg to males and 300, 640, 1,260, 2,600, or 5,300 mg/kg to females) for 14 weeks. All mice survived until the end of the study. Mean body weights and feed consumption were similar among exposed and control groups. A responsive anemia occurred in exposed mice at week 14. Liver weights of exposed groups of mice were significantly greater than those of the control groups.

The incidences of centrilobular hypertrophy in the liver of mice exposed to 3,750 ppm or greater were significantly greater than those in the controls, and the severities increased with increasing exposure concentration. Cytoplasmic alteration of the urinary bladder was observed in all exposed mice, and the severities increased with increasing exposure concentration. The incidences of hematopoietic cell proliferation were increased in all exposed groups of males and females, and pigmentation was observed in the spleen of all exposed mice (except one male and one female in the 30,000 ppm groups).

2-Year Study in Rats

Groups of 50 male and 50 female F344/N rats were fed diets containing 469, 938, or 1,875 ppm anthraquinone for 105 weeks. Groups of 60 male and 60 female F344/N rats received 0 or 3,750 ppm anthraquinone for the same period. Five males and five females receiving 0 or 3,750 ppm were evaluated for histopathology and α2u-globulin concentrations at 3 months and for organ weights and histopathology at 12 months. Dietary concentrations of 469, 938, 1,875, and 3,750 ppm anthraquinone resulted in average daily doses of approximately 20, 45, 90, and 180 mg/kg to males and 25, 50, 100, and 200 mg/kg to females. Additional groups of 18 males given 469, 938, 1,875, or 3,750 ppm for 8 days and 10 males and 10 females given 469, 938, or 1,875 ppm for 3, 6, 12, or 18 months were designated for toxicokinetic studies.

Survival, Body Weights, and Feed Consumption

Survival of exposed groups of males was similar and survival of exposed groups of females was greater than that of the controls. Mean body weights of exposed groups of males during the latter part of the study and mean body weights of exposed females throughout most of the study were less than those of the controls. Feed consumption by exposed groups was similar to that by the controls.

Pathology Findings

The incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) occurred with positive trends and were increased in all exposed groups of female rats. The incidences of renal tubule adenoma in all exposed groups of male rats exceeded the historical control range, and the incidence was significantly increased in the 938 ppm group. Increased incidences of nonneoplastic lesions of the kidney associated with anthraquinone exposure included hyaline droplet accumulation, pigmentation, and mineralization in the renal medulla and transitional epithelial hyperplasia in males and females and renal tubule hyperplasia in females. Incidences of nephropathy were increased in females, and severities of nephropathy were increased in males. At 3 months, the concentration of α2u-globulin in the kidney of 3,750 ppm males was greater than that in the control group.

The incidence of urinary bladder transitional epithelial papilloma in 1,875 ppm males was significantly greater than that in the control group, and the incidences in groups of males exposed to 938 ppm or greater exceeded the historical control range. There were positive trends in the incidences of transitional epithelial hyperplasia and papilloma or carcinoma (combined) of the urinary bladder in females.

The incidences of hepatocellular adenoma or carcinoma (combined) were slightly increased in exposed males and females; the incidences in groups of females exposed to 938 ppm or greater exceeded the historical control range. The incidences of several nonneoplastic liver lesions of minimal severity were also increased.

The incidences of congestion, pigmentation, and hematopoietic cell proliferation of the spleen were greater in exposed males and females than in the controls. The incidences of bone marrow hyperplasia were increased in most groups of exposed rats, and the incidences of bone marrow atrophy were increased in exposed females.

The incidences of mononuclear cell leukemia in all exposed groups were significantly less than those in the controls at 2 years, and the incidences were less than the historical control ranges.

2-Year Study in Mice

Groups of 50 male and 50 female B6C3F$_1$ mice were fed diets containing 0, 833, 2,500, or 7,500 ppm anthraquinone (equivalent to average daily doses of approximately 90, 265, or 825 mg/kg to males and 80, 235, or 745 mg/kg to females) for 105 weeks. Additional groups of 36 males given 833, 2,500, or 7,500 ppm for 8 days and 10 males and 10 females given 833, 2,500, or 7,500 ppm for 12 months were designated for toxicokinetic studies.

Survival, Body Weights, and Feed Consumption

Survival of 7,500 ppm males was less than that of the control group. Mean body weights of 7,500 ppm males during the last 6 months of the study and mean body weights of 7,500 ppm females at the end of the study were less than those of the control groups. Feed consumption by all groups of males and females was similar to that by the controls.

Pathology Findings

Incidences of hepatocellular neoplasms (including multiple neoplasms) increased with a positive trend in male and female mice, and the incidences were increased in all exposed groups. Incidences of hepatoblastoma were significantly increased in males exposed to 2,500 or 7,500 ppm. The incidences of several nonneoplastic lesions of the liver were increased in exposed mice. There was a marginal increase in the incidences of neoplasms of thyroid gland follicular cells in males and females. Incidences of intracytoplasmic inclusion body of the urinary bladder and hematopoietic cell proliferation of the spleen in

males and females and thyroid gland follicular cell hyperplasia and kidney pigmentation in males were greater in exposed groups than in the controls.

GENETIC TOXICOLOGY

Anthraquinone (97% pure) was mutagenic in *S. typhimurium* strains TA98 and TA100, with and without rat and hamster S9 metabolic activation enzymes. A second *Salmonella* test with a 100% pure anthraquinone sample showed no mutagenic activity in strains TA98, TA100, or TA102, with or without rat liver S9 enzymes. Several substituted anthraquinones were also tested in *Salmonella*, and results showed significant mutagenic activity for 2-hydroxyanthraquinone and 1-, 2-, and 9-nitroanthracene, with and without S9. 1-Hydroxyanthraquinone was not mutagenic in *Salmonella*, with or without S9. Significant increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood samples from male and female mice exposed to anthraquinone (99.9% pure) in feed for 14 weeks. However, results of an acute exposure mouse bone marrow micronucleus test, with anthraquinone administered by intraperitoneal injection, were negative.

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

A physiologically based pharmacokinetic model resulting from oral exposure was developed to characterize tissue concentrations of anthraquinone in rats. Data used to create the model were obtained from the literature or from the current studies. The physiologically based pharmacokinetic model indicates that anthraquinone is slowly and incompletely absorbed, distributed slowly to tissues by a diffusion-limited transport process, stored in fatty tissues, and is slowly metabolized. Model-based plasma anthraquinone concentrations may serve as a surrogate dosimeter for evaluating neoplasm exposure concentration-response data.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *some evidence of carcinogenic activity** of anthraquinone in male F344/N rats based on increased incidences of renal tubule adenoma and of transitional epithelial papillomas of the kidney and urinary bladder. Hepatocellular neoplasms may have been related to exposure to anthraquinone. There was *clear evidence of carcinogenic activity* of anthraquinone in female F344/N rats based on increased incidences of renal tubule neoplasms. Increases in the incidences of urinary bladder transitional epithelial papilloma or carcinoma (combined) and of hepatocellular adenoma in female rats were also related to anthraquinone exposure. There was *clear evidence of carcinogenic activity* in male and female $B6C3F_1$ mice based on increased incidences of liver neoplasms. Thyroid gland follicular cell neoplasms in male and female mice may have been related to anthraquinone exposure.

Exposure to anthraquinone for 2 years caused increases in the incidences of nonneoplastic lesions of the kidney, liver, spleen, and bone marrow in male and female rats, the liver, urinary bladder, and spleen in male and female mice, and the thyroid gland and kidney in male mice.

Decreased incidences of mononuclear cell leukemia in male and female rats were attributed to exposure to anthraquinone.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report from May 21, 1999, appears on page 17.

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Concentrations in feed	0, 469, 938, 1,875, or 3,750 ppm	0, 469, 938, 1,875, or 3,750 ppm	0, 833, 2,500, or 7,500 ppm	0, 833, 2,500, or 7,500 ppm
Body weights	Exposed groups less than control group	Exposed groups less than control group	7,500 ppm group less than control group	7,500 ppm group slightly less than control group
Survival rates	22/50, 23/50, 22/50, 26/50, 22/50	23/50, 40/50, 35/50, 37/50, 40/50	45/50, 41/50, 43/50, 23/50	35/50, 42/50, 35/50, 42/49
Nonneoplastic effects	Kidney: hyaline droplet accumulation (3/50, 14/50, 10/50, 16/50, 16/50); severity of nephropathy (2.2, 3.1, 3.1, 3.0, 3.0); pigmentation (25/50, 31/50, 36/50, 38/50, 33/50); medulla, mineralization (30/50, 42/50, 46/50, 47/50, 49/50); transitional epithelium, hyperplasia (28/50, 45/50, 44/50, 48/50, 48/50) Liver: centrilobular hypertrophy (0/50, 4/50, 21/50, 13/50, 29/50); cystic degeneration (9/50, 31/50, 36/50, 28/50, 29/50); inflammation (13/50, 30/50, 28/50, 30/50, 27/50); eosinophilic focus (9/50, 22/50, 30/50, 29/50, 20/50); mixed cell focus (4/50, 12/50, 15/50, 13/50, 10/50); cytoplasmic vacuolization (5/50, 18/50, 23/50, 17/50, 23/50) Spleen: congestion (6/50, 35/50, 37/50, 30/50, 31/50); pigmentation (12/50, 36/50, 38/50, 33/50, 28/50); hematopoietic cell proliferation (37/50, 45/50, 44/50, 43/50, 39/50)	Kidney: hyaline droplet accumulation (33/50, 48/50, 45/50, 44/50, 44/49); nephropathy (39/50, 49/50, 47/50, 49/50, 49/49); pigmentation (27/50, 50/50, 48/50, 50/50, 47/49); medulla, mineralization (17/50, 25/50, 27/50, 28/50, 20/49); renal tubule, hyperplasia (0/50, 12/50, 13/50, 15/50, 11/49); transitional epithelium, hyperplasia (0/50, 5/50, 12/50, 3/50, 10/49) Liver: centrilobular hypertrophy (0/50, 18/50, 23/50, 19/50, 26/49); cystic degeneration (0/50, 5/50, 10/50, 10/50, 6/49); inflammation (25/50, 46/50, 44/50, 38/50, 46/49); eosinophilic focus (8/50, 32/50, 34/50, 39/50, 34/49); mixed cell focus (3/50, 30/50, 20/50, 23/50, 13/49); angiectasis (3/50, 15/50, 18/50, 15/50, 21/49) Spleen: congestion (1/50, 46/50, 42/50, 44/50, 45/49); pigmentation (33/50, 45/50, 48/50, 48/50, 47/49); hematopoietic cell proliferation (39/50, 50/50, 47/50, 47/50, 46/49)	Liver: centrilobular, hypertrophy (24/50, 34/50, 41/50, 33/49); degeneration, fatty, focal (0/50, 7/50, 6/50, 0/49); hepatocyte, erythrophagocytosis (1/50, 9/50, 13/50, 6/49); eosinophilic focus (14/50, 17/50, 24/50, 20/49); focal necrosis (2/50, 3/50, 3/50, 8/49) Urinary Bladder: intracytoplasmic inclusion body (0/50, 46/49, 46/49, 42/45) Thyroid Gland: follicular cell hyperplasia (7/50, 10/50, 15/49, 21/46) Spleen: hematopoietic cell proliferation (12/50, 14/50, 12/49, 30/42) Kidney: pigmentation (0/50, 2/50, 2/50, 18/47)	Liver: centrilobular hypertrophy (1/49, 27/50, 22/50, 39/49); degeneration, fatty, focal (2/49, 3/50, 1/50, 9/49); eosinophilic focus (6/49, 15/50, 11/50, 22/49) Urinary Bladder: intracytoplasmic inclusion body (0/44, 40/48, 43/46, 46/48) Spleen: hematopoietic cell proliferation (9/45, 17/49, 17/48, 26/48)

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Nonneoplastic effects (continued)	Bone Marrow: hyperplasia (25/50, 28/50, 37/50, 36/50, 33/50)	Bone Marrow: hyperplasia (19/50, 31/50, 28/50, 19/50, 23/50); atrophy (4/50, 13/50, 13/50, 11/50, 13/50)		
Neoplastic effects	Kidney: renal tubule adenoma (1/50, 3/50, 9/50, 5/50, 3/50); transitional epithelial papilloma (0/50, 0/50, 2/50, 0/50, 1/50) Urinary Bladder: transitional epithelial papilloma (0/50, 1/50, 3/50, 7/50, 3/49)	Kidney: renal tubule adenoma (0/50, 4/50, 9/50, 7/50, 12/49); renal tubule adenoma or carcinoma (0/50, 6/50, 9/50, 8/50, 14/49) Urinary Bladder: transitional epithelial papilloma or carcinoma (0/49, 0/49, 0/49, 1/50, 2/49) Liver: hepatocellular adenoma (0/50, 2/50, 6/50, 4/50, 3/50)	Liver: hepatocellular adenoma (21/50, 32/50, 38/50, 41/49); hepatocellular carcinoma (8/50, 13/50, 17/50, 21/49); hepatoblastoma (1/50, 6/50, 11/50, 37/49); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (26/50, 35/50, 43/50, 48/49)	Liver: hepatocellular adenoma (6/49, 28/50, 27/50, 40/49); hepatocellular carcinoma (2/49, 3/50, 8/50, 8/49); hepatocellular adenoma or carcinoma (6/49, 30/50, 30/50, 41/49)
Equivocal findings	Liver: hepatocellular adenoma or carcinoma (1/50, 3/50, 4/50, 5/50, 3/50)	None	Thyroid Gland: follicular cell adenoma (0/50, 0/50, 2/49, 2/46)	Thyroid Gland: follicular cell adenoma (1/45, 1/48, 2/48, 2/48); follicular cell carcinoma (0/45, 0/48, 0/48, 2/48); follicular cell adenoma or carcinoma (1/45, 1/48, 2/48, 4/48)
Decreased incidences	Mononuclear Cell Leukemia: (25/50, 2/50, 1/50, 5/50, 7/50)	Mononuclear Cell Leukemia: (18/50, 1/50, 1/50, 2/50, 0/50)	None	None
Level of evidence of carcinogenic activity	Some evidence	Clear evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:				
Anthraquinone (97% pure)		Positive in strains TA98 and TA100 with and without S9		
Anthraquinone (100% pure)		**Negative in strains TA98, TA100, and TA102 with and without S9**		
1-Hydroxyanthraquinone		**Negative in strains TA98, TA100, and TA102 with and without S9**		
2-Hydroxyanthraquinone		**Positive in strain TA98 without S9, negative in strains TA98 with S9 and TA100 with and without S9**		
1-Nitroanthracene		**Positive in strains TA98 and TA100 with and without S9**		
2-Nitroanthracene		**Positive in strains TA98 and TA100 with and without S9**		
9-Nitroanthracene		**Positive in strains TA98 and TA100 with and without S9**		
Micronucleated erythrocytes				
Mouse bone marrow *in vivo*:		Negative		
Mouse peripheral blood *in vivo* (99.9% pure anthraquinone):		Positive		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

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.

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on anthraquinone on May 21, 1999, are listed below.

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* Did not attend

The members of the Technical Reports Review Subcommittee who evaluated the revised draft NTP Technical Report on anthraquinone on February 18, 2004, are listed below.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On May 21, 1999, the draft Technical Report on the toxicology and carcinogenesis studies of anthraquinone received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review 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 anthraquinone by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on the survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in male and female rats and mice. The proposed conclusions for the 2-year studies were *some evidence of carcinogenic activity* in male F344/N rats, *clear evidence of carcinogenic activity* in female F344/N rats, and *clear evidence of carcinogenic activity* in male and female B6C3F$_1$ mice.

Dr. Irwin reported that the metabolism of anthraquinone is extremely complicated and described short-term studies in rats that measured activities in the liver of two cytochrome P450 enzymes and levels of 8-hydroxy-2′-deoxyguanosine in liver, kidney, and urinary bladder. While there was only modest induction of the hepatic activity of ethoxyresorufin-*O*-dealkylase (P4501A1) activity, there was a strong induction of pentoxyresorufin-*O*-dealkylase (P4502B1) activity. This amounted to about an 80-fold increase over control in male rats and about a 40-fold increase over control in female rats. Dr. Irwin stated that cell proliferation was measured in liver, kidney, and urinary bladder after administration of BrdU in drinking water. There was no increased proliferation in liver or kidney in rats, but there were moderate increases in the urinary bladder. Dr. Irwin compared neoplastic findings from anthraquinone, the parent compound, to findings from six substituted anthraquinone derivatives studied by the NTP. He concluded that the parent ring system confers carcinogenic

potential while the various substituents play a major role in determining the target organs affected and the strength of the carcinogenic response.

Dr. C.J. Portier, NIEHS, presented data on the toxicokinetics (TK) of anthraquinone, noting that standard TK protocols were run along with measurements of biliary anthraquinone concentrations after a single intravenous injection in male rats. The model used was a standard physiologically based pharmacokinetic model for highly lipophilic compounds. Dr. Portier said that there did not appear to be a first pass effect in the liver; absorption was directly into venous blood, while distribution was through a restricted capillary:tissue transport mechanism. Metabolism was through inducible Michaelis-Menton kinetics in the liver. Elimination is through urinary and biliary excretion of parent and metabolites with some enterohepatic cycling. Dr. Portier presented graphics comparing actual data points with those predicted by the model. He summarized conclusions drawn from the TK data. First there is delayed absorption and very slow clearance. In the female rat, there are higher tissue concentrations due to slow clearance and slower metabolism of anthraquinone. Transport is diffusion-limited in most tissues. There is markedly slower absorption from feed than from gavage dosing. Finally, chronic exposure induces metabolism of the parent compound.

Dr. Medinsky, a principal reviewer, agreed with the proposed conclusions. She thought the pharmacokinetics supported the conclusion for carcinogenicity and provided an adjunct to our understanding, especially with regard to why there was *clear evidence* in female but not male rats. Dr. Medinsky commented that it was difficult to adequately evaluate the model because of lack of explanatory text regarding assumptions underlying the model.

Dr. Cullen, the second principal reviewer, agreed with the proposed conclusions. He asked for clarification of the α2u-globulin protein droplet renal injury in the 14-week studies in male rats and its relationship to risk of tumor development in the 2-year studies. Dr. J.R. Hailey, NIEHS, responded

that if a significant amount of α2u-globulin occurs in the kidney with angular crystals and an increase in renal tumors, then the mode of action seems fairly well described. However, this would not explain the increased incidence of renal tumors in female rats, which do not secrete much α2u-globulin, and therefore, he did not think it possible to sort out what part of the kidney tumor effect in males might be related to α2u nephropathy and what part might be related to a mechanism of action operative in females. Dr. Cullen asked for discussion on the rationale for setting higher chronic doses in mice than in rats and whether this may have impacted the incidence of hepatocellular tumors in rats. Dr. Irwin commented that nephropathy is always a major consideration for setting doses in rats, and that it and increases in hepatocellular hypertrophy in the 14-week study were the major determinants for selecting doses in rats, while increases in mouse liver weights, as much as 30% at the highest dose in the 14-week study, were a major factor in setting doses for mice.

Dr. Russo, the third principal reviewer, agreed with the proposed conclusions.

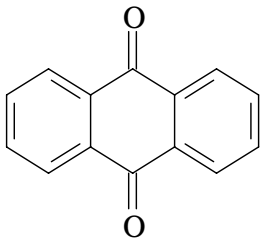
In further discussion about the dose setting, Dr. Davis asked if the lack of 14-day studies may have resulted in doses for the 14-week and 2-year studies that were not low enough; that is, there was no dose in the 2-year studies at which increased tumor incidences were not seen. He wondered what this meant with regard to human exposure. Dr. Russo observed that there was a clear dose response. Dr. Davis agreed, but said it was still helpful to have a no-effect-level. Dr. Carlson suggested that a better explanation of how the doses were set is needed. Dr. Irwin said that there is always an attempt to reach a no-effect-level.

Dr. Medinsky moved that the Technical Report on anthraquinone be accepted with the revisions discussed and the conclusions as written for male and female rats and female mice and that the conclusion for male mice be modified to include the statement that renal tubule neoplasms may have

been related to anthraquinone exposure. Dr. Cullen seconded the motion, which was accepted unanimously with nine votes.

Subsequent to the review of the draft Technical Report on anthraquinone, J.A. Cook, Technical Director, Chemical Products Corporation, Cartersville, GA, suggested to the NTP that the 0.1% contaminant in the anthraquinone studies might contain the mutagen 9-nitroanthracene and that this might account for the carcinogenicity of the tested material. He also stated that this contaminant would not be present in anthraquinone manufactured by processes other than oxidation of anthracene. In response, the NTP agreed to clarify the process used to manufacture the anthraquinone used in its studies, examine the issue of the mutagenicity of anthraquinone and its metabolites and contaminants, and revise the discussion of the Technical Report to address the potential impact of the findings on the interpretation of the 2-year studies. As a consequence, the revised Technical Report was brought before the NTP Technical Reports Review Subcommittee on November 5, 2003, for reconsideration.

INTRODUCTION



ANTHRAQUINONE

CAS No. 84-65-1

Chemical Formula: $C_{14}H_8O_2$ Molecular Weight: 208.22

Synonyms: 9,10-Anthracenedione; anthradione; 9,10-anthraquinone; 9,10-dioxoanthracene; 9,10-dihydro-9,10-dioxoanthracene
Trade names: Corbit, Hoelite, Morkit

CHEMICAL AND PHYSICAL PROPERTIES

Anthraquinone is a golden yellow, crystalline powder with a slight odor. It is soluble in alcohol, toluene, and hot benzene, moderately soluble in ethanol, slightly soluble in ether, and insoluble in acetone and water. Anthraquinone has a boiling point of approximately 377° C at 760 mm Hg and has a specific gravity of 1.438 at 20° C, a vapor pressure of 1 mm at 190° C, and a vapor density of 7.16. Anthraquinone has a flash point of 185° C and is flammable when exposed to heat or flame. When heated to decomposition, it emits acrid smoke and irritating fumes (*Hawley's*, 1997; Lewis, 1997).

PRODUCTION, USE, AND HUMAN EXPOSURE

Several methods have been used to manufacture anthraquinone (*Kirk-Othmer,* 1978). Anthracene can be oxidized to anthraquinone with sodium dichromate in sulfuric acid. Anthraquinone has also been manufactured in a Friedel-Crafts reaction involving the reaction of thalic anhydride with benzene in the

presence of aluminum chloride to produce *o*-benzoylbenzoic acid, which is then cyclized to anthraquinone. A more recently developed process involved a Diels-Alder addition of butadiene to naphthoquinone followed by oxidation of the resulting tetrahydroanthraquinone to 9,10-anthraquinone.

Although current production figures are not available, foreign trade statistics compiled by the U.S. Census Bureau (1997) indicate a total of 11,727,320 kg of anthraquinone was imported to the United States while 2,279,258 kg were exported in 1997.

Anthraquinone is used as an intermediate in the manufacture of dyes and pigments as well as numerous other organic compounds. For the production of dyes, anthraquinone undergoes substitution with amino and halide groups to alter color and facilitate additional derivatization. Anthraquinone dyes are particularly useful because of their fastness, and they are found in all classes of applications such as disperse and mordant dyes.

Anthraquinone has been used in the pulp and paper industry as an additive in the kraft pulping process (Voss, 1981). In this application, anthraquinone is added directly to the strong alkaline solution of sodium hydroxide and sodium sulfide that is used to separate cellulose and hemicellulose fibers and to degrade lignin. Although finished paper may contain small quantities of anthraquinone, the major problem is removal of anthraquinone from the aqueous effluent prior to wastewater discharge.

Anthraquinone has also been used as a catalyst in the isomerization of vegetable oils, an accelerator in nickel electroplating, and as a bird repellant sprayed on growing crops or applied as a seed dressing (Meister, 1987). **More recently, anthraquinone has been widely used as a bird repellent around airport runways (Ballinger *et al.*, 1988; Ballinger and Price, 1996).**

The National Occupational Health Survey conducted by the National Institute for Occupational Safety and Health from 1972 to 1974 estimated that 2,202 workers in 81 plants were potentially exposed to

anthraquinone in the workplace based on the observation of the use of anthraquinone or the use of trade name products containing anthraquinone (NIOSH, 1976). A second workplace survey conducted from 1980 to 1983 indicated that 28 workers were exposed at seven sites. These latter reports were based only on direct observation by the surveyor of the actual use of anthraquinone (NIOSH, 1990).

Anthraquinone has been identified in atmospheric samples (Cautreels *et al.,* 1982), diesel engine exhaust (Yu and Hites, 1981; Choudhury, 1982; Ciccioli *et al.,* 1986), samples of fly ash collected from municipal incinerators (Eiceman *et al.,* 1979), and in surface waters (Meijers and Van der Leer, 1974), tap water (Akiyama *et al.,* 1980), and finished drinking water sampled from 12 municipalities on the Great Lakes (Williams *et al.,* 1982).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Published information on the metabolism of anthraquinone is very limited. Sato *et al.* (1956) identified 1-hydroxyl and 2-hydroxyl anthraquinone in the urine of rats that received daily oral doses of anthraquinone. Sims (1964) administered diets containing 5% anthraquinone to four male Chester-Beatty rats for 4 days. Analysis of pooled urine samples collected over the 4 days revealed the presence of the sulfate and glucuronide conjugates of 2-hydroxyanthraquinone, 9,10-dihydroxy-anthracene, and 2,9,10-trihydroxyanthracene.

In studies conducted as part of the current studies, the metabolism and disposition of anthraquinone in male F344/N rats were examined. Animals were administered uniformly labeled ^{14}C-anthraquinone by intravenous injection at 0.35 mg/kg body weight or by gavage at doses ranging from 0.35 to 350 mg/kg. Following oral administration, anthraquinone was absorbed from the gastrointestinal tract and distributed to tissues. Although the highest concentration of anthraquinone was initially found in adipose tissue, no

indication of bioaccumulation was apparent in any tissue. The majority of the radiolabel was eliminated in the feces and the urine by 24 hours after dosing at all four concentrations. At 96 hours after dosing, less than 5% of the administered dose remained in major tissues (Appendix N).

Elimination of over 50% of the administered radioactivity in the feces suggested substantial excretion of parent and/or metabolites in the bile. This was confirmed by administering anthraquinone to bile-duct cannulated rats. During the 6-hour period of sample collection, 35% of the administered dose was recovered in bile. Analysis of the bile samples indicated that less than 3% of the radioactivity collected was present as the parent compound, suggesting extensive hepatic metabolism. Analysis of urine from dosed rats by high performance liquid chromatography revealed the presence of as many as 11 metabolites. Two of the metabolites identified were 1-hydroxyanthraquinone and 2-hydroxyanthraquinone.

Humans

No information on the absorption, distribution, metabolism, or excretion of anthraquinone in humans was found in a review of the available literature.

TOXICITY

Experimental Animals

There is very little published information on the subchronic toxicity of anthraqinone. The *Registry of Toxic Effects of Chemical Substances* (1998) lists the LD_{50} as 3,500 mg/kg for intraperitoneal administration to rats, the LC_{50} as 1,300 mg/m^3 inhalation for rats, and the oral LD_{50} as 5 g/kg for mice. Pidemskii and Masenko (1970; cited in ITC information review) reported hepatotoxicity in rats that received daily oral doses of 50 mg/kg for 7 days. Volodchenko and Labunskii (1972) observed reduced hemoglobin concentrations and erythrocyte counts in rats exposed to atmospheres containing 12.2 mg/m^3 anthraquinone for an unspecified period.

Humans

No information on the toxicity of anthraquinone in humans was found in a review of the available literature.

CARCINOGENICITY

Experimental Animals

The carcinogenic potential of anthraquinone was evaluated by Innes *et al.* (1969) in two strains of mice. At 7 days of age and continuing through 28 days of age, groups of 18 male or 18 female B6C3F$_1$ or B6AKRF$_1$ mice received 464 mg anthraquinone/kg body weight daily by gavage. After 28 days, these groups received 1,206 ppm anthraquinone in feed for 18 months. Although actual data were not shown, no increase in tumors in either strain of mice was associated with administration of anthraquinone.

Several substituted anthraquinones have been evaluated for carcinogenic potential. 2-Aminoanthraquinone was administered in feed at concentrations of 0%, 1%, 3%, or 5% to groups of 50 male or 50 female F344/N rats or B6C3F$_1$ mice for 78 weeks. At the end of the exposure period, animals were switched to control feed. Rats were held for an additional 32 weeks and mice for 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 to groups of 50 male or 50 female F344/N rats or B6C3F$_1$ mice. Rats received 0%, 0.03%, or 0.06% 1-amino-2-methylanthraquinone for 17 weeks and then 0.12% or 0.24% for 62 weeks, followed by a 28-week observation period during which control feed was provided. Mice received 0%, 0.03%, or 0.06% for 17 weeks after which the group receiving 0.03% was given feed containing 0.12% for the remainder of the exposure period. Exposure to 1-amino-2-methylanthraquinone significantly increased incidences of hepatocellular neoplasms in male and female rats and mice and renal neoplasms in male rats (NCI, 1978b).

2-Methyl-1-nitroanthraquinone was administered in feed to groups of 50 male or 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 given control feed. Significant increases in the incidences of hepatocellular neoplasms in male and female rats were 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 or 50 female F344/N rats at dietary concentrations of 0, 1,250, 2,500, or 5,000 ppm and to groups of 50 male or 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 of 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 or 50 female F344/N rats or $B6C3F_1$ 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 neoplasms of the forestomach, liver, and lung in mice (NTP, 1996).

Humans

No information on the carcinogenicity of anthraquinone in humans was found in a review of the available literature.

GENETIC TOXICITY

Mutagenic activity of anthraquinone has been demonstrated both *in vitro* and *in vivo*, although much of the observed activity has been attributed to contaminants, depending upon the method used to produce the anthraquinone under study. Early mutagenicity studies of anthraquinone in *Salmonella typhimurium,* most using the plate incorporation assay protocol, reported negative results (Brown and Brown, 1976; Anderson and Styles, 1978; Gibson *et al.,* 1978; Salamone *et al.,* 1979; Tikkanen *et al.,* 1983; Sakai *et al.,* 1985). Later studies showed clear mutagenic activity for anthraquinone in TA100 and the frameshift strains TA98, TA1537, and TA1538, in the presence and absence of S9 activation enzymes (Liberman *et al.,* 1982; Zeiger *et al.,* 1988). None of the bacterial mutagenicity assays that reported negative results included the purity of the anthraquinone samples used for testing. The Zeiger *et al.* (1988) preincubation assay that produced positive results tested an anthraquinone sample that was 97% pure. Sample purity, along with dose selection and other protocol variations, may have been critical to the outcome of these mutagenicity assays. A structurally related compound, 9-nitroanthracene, which was present at about 0.12% in the anthraquinone sample tested by Zeiger *et al.* (1988), was positive in the *Salmonella* mutation assay over a concentration range of 10 to 1,000 μg/plate using strains TA98 and TA100, with and without 30% hamster or rat liver S9 activation enzymes (Zeiger *et al.*, 1988). A number of investigators have studied the mutagenicity of substituted anthraquinones in the *Salmonella* assay and have suggested that certain methyl, nitro, and phenolic substitutions confer enhanced mutagenic activity after metabolic activation (Brown and Dietrich, 1979; Fu *et al.*, 1986; Krivobok *et al.*, 1992). Hydroxylation, up to a maximum of four substitutions, also appears to enhance mutagenic potential (Tikkanen *et al.*, 1983; Matsushima *et al.*,

1986). Thus, particular substituted compounds appear to be more mutagenically active than the parent compound, anthraquinone.

A recent study reported results of a *Salmonella* mutation assay using the same aliquot of anthraquinone that was tested in the 2-year bioassays presented in this Technical Report (99% pure) (Butterworth *et al.*, 2001). The authors concluded that, although the chemical produced a positive response in strains TA98, TA100, and TA1537 with and without S9, the observed mutagenicity was the result of a low level of 9-nitroanthracene present as a contaminant in the sample. To further support this hypothesis, the authors purified the anthraquinone sample, retested it along with anthraquinone samples produced by chemical processes not believed to result in appreciable contamination, and observed no indication of mutagenic activity in any of these samples. Therefore, they concluded that the mutagenic activity displayed by original, 99% pure sample was produced by the contaminant 9-nitroanthracene.

Two identified rat metabolites of anthraquinone, anthrone and 2-hydroxyanthraquinone, were reported to be weak mutagens in *S. typhimurium* (Tikkanen *et al.*, 1983; Moller *et al.*, 1985; Ramdahl, 1985; Matsushima *et al.*, 1986). As with anthraquinone, anthrone was reported to lack mutagenicity in several studies (Brown and Brown, 1976; Anderson and Styles, 1978; Gibson *et al.*, 1978; Liberman *et al.*, 1982). Thus, protocol characteristics, dose, and purity may all be important factors in the detection of mutagenicity of anthraquinone and substituted anthraquinones. In addition, the identity of specific side groups and their spatial orientation to the main ring structure of the anthraquinone molecule are important to the mutagenic activity of the chemical.

Cesarone *et al.* (1982) reported *in vivo* induction of DNA strand breaks by anthraquinone in liver and kidney cells of CD-1 mice treated with 250 mg anthraquinone/kg via intraperitoneal injection, and dose-related increases in micronuclei were reported in cultured Syrian hamster embryo cells treated

with 3.13 to 25 μg anthraquinone (99% pure)/mL (Gibson *et al.,* 1997). However, when anthraquinone was tested for induction of forward mutations in cultured human BT lymphoblastoid cells, a metabolically competent cell line for polycyclic aromatic compounds, no mutagenic activity was detected (Durant *et al.,* 1996). Butterworth *et al.* (2001) reported negative results with anthraquinone produced through a Diels-Alder process in an acute mouse bone marrow micronucleus test and in a mouse lymphoma L5178Y cell forward mutation assay.

STUDY RATIONALE

Anthraquinones form a large class of commercially important chemicals and constitute the largest class of naturally occurring quinones. Because of the ubiquity of compounds containing the anthraquinone ring system, the National Toxicology Program has been involved in a class study of these compounds. In previous studies, five substituted anthraquinones have exhibited significant carcinogenic potential in long-term rodent studies. Anthraquinone, the parent compound, was selected for this class study to aid in understanding the impact on the carcinogenic response by various substitutions to the anthraquinone ring and because its use pattern suggests the potential for human exposure.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF ANTHRAQUINONE

Anthraquinone was obtained from Zeneca Fine Chemicals (Wilmington, DE) in one lot (5893). Identity, purity, and stability analyses were conducted by the study laboratory (Appendix J). Reports on analyses performed in support of the anthraquinone studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a golden yellow crystalline powder, was identified as anthraquinone by infrared and proton nuclear magnetic resonance spectroscopy. The purity of lot 5893 was determined by gas chromatography. The results indicated a purity of approximately **99.9% (which was consistent with the manufacturer's specifications). Additional purity analysis by high-performance liquid chromatography/ultraviolet detection indicated a purity greater than 99.5%.**

Stability studies of the bulk chemical were performed by the study laboratory using gas chromatography. These studies indicated that anthraquinone was stable as a bulk chemical for up to 2 weeks when stored in sealed containers protected from ultraviolet light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in amber glass bottles with Teflon-lined caps. Stability was monitored during the 14-week and 2-year studies using gas chromatography. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared every 4 weeks by mixing anthraquinone with feed (Table J1). Formulations were stored in polyethylene bags in sealed plastic buckets at room temperature for up to 35 days. Homogeneity studies of the 1,875 and 30,000 ppm dose formulations and stability studies of a 230 ppm formulation were performed by the study laboratory using gas chromatography. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 35 days for dose formulations stored at room temperature in sealed containers protected from light and for 7 days at room temperature exposed to air and light.

Periodic analyses of the dose formulations of anthraquinone were conducted at the study laboratory using gas chromatography. Dose formulations were analyzed at the beginning and end of the 14-week studies (Table J2) and approximately every 8 or 12 weeks for the 2-year studies (Table J3). Of the dose formulations analyzed during the 14-week and 2-year studies, all dose formulations were within 10% of the target concentrations. For the 14-week studies, four of five animal room samples for rats and nine of ten for mice were within 10% of the target concentrations. All animal room samples for rats and eight of twelve for mice in the 2-year studies were within 10% of the target concentrations.

14-WEEK STUDIES

The 14-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to anthraquinone and to determine the appropriate doses 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, rats and mice were approximately 4 weeks old. Rats were quarantined for 11 (males) or 12 (females) days and mice were quarantined for 13 (males) or 14 (females) days; rats and mice were

approximately 6 to 7 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. Serologic analyses were performed on up to five male and five female sentinel rats and mice 1 month after study start and on up to five male and five female control rats and sentinel mice at study termination 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, 1,875, 3,750, 7,500, 15,000, or 30,000 ppm anthraquinone for 14 weeks. Clinical pathology study groups of 10 male and 10 female rats received the same concentrations of anthraquinone for 25 days. Water was available *ad libitum* and feed was available *ad libitum* except during urine collection periods. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded and animals were weighed weekly and at the end of the studies. Feed consumption was recorded twice weekly or once weekly (male mice). Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected from the retroorbital sinus of clinical pathology study rats under carbon dioxide anesthesia on days 4 and 22. Using the same method, blood was collected from all core study rats and mice surviving to the end of the studies for hematology and clinical chemistry (rats) analyses. Blood samples for hematology analyses were placed into microcollection tubes containing potassium EDTA. Erythrocyte, platelet, and leukocyte counts, hematocrit values, hemoglobin concentration, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration were determined using a Serono-Baker System 9000 hematology analyzer (Serono-Baker Diagnostics, Allentown, PA). Differential leukocyte counts and erythrocyte and platelet morphologies were determined microscopically from blood smears stained with Wright-Giemsa stain on a Hema-Tek slide stainer (Miles Laboratory, Ames Division, Elkhart, IN). A Miller disc was used to determine reticulocyte counts from smears prepared with blood stained with new methylene blue. For clinical chemistry analyses, blood samples from rats were placed into microcollection serum separator tubes, centrifuged, and the serum samples were analyzed using a Hitachi 704® chemistry

analyzer (Boehringer Mannheim, Indianapolis, IN) using commercially available reagents. The hematology and clinical chemistry parameters measured are listed in Table 1.

Urine samples were collected from clinical pathology study rats on days 8 and 26 and from core study rats on day 89. All rats were placed in metabolism cages for 16 hours, and urine collection tubes were placed in an ice bath during collection. Clinical pathology study rats were discarded without necropsy following the day 26 urine collection period. Urine total volume was measured, and specific gravity was determined using an American Optical Refractometer/Total Solids Meter (American Optical, Buffalo, NY). All other urine parameters were determined using a Hitachi 704® analyzer. 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 on core study rats and mice exposed to 0, 7,500, 15,000, and 30,000 ppm. 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, 1992). 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% dimethylsulfoxide. 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 4 to 6 μm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all core study 0 and 30,000 ppm rats and mice. Table 1 lists the tissues and organs routinely examined.

At necropsy, right kidneys of male rats were bisected transversely. The caudal half was placed in a vial containing phosphate-buffered saline and frozen. Before analysis, each sample was defrosted, the buffer was removed and replaced with a sodium/potassium buffer, the sample was homogenized, and the supernatant was drawn off. Soluble protein content was measured in a 1:50 dilution with phosphate-buffered saline/Tween using a pyrogallol assay. Concentrations of α2u-globulin were measured using a validated enzyme-linked immunosorbent assay (Fuciarelli *et al.,* 1996). Parameters measured are listed in Table 1.

2-YEAR STUDIES

Study Design

Groups of 60 male and 60 female rats were fed diets containing 0 or 3,750 ppm anthraquinone, and 50 male and 50 female rats received 469, 938, or 1,875 ppm anthraquinone for 105 weeks. Five male and five female 0 and 3,750 ppm rats were evaluated at 3 and 12 months. Additional groups of 18 male rats given 469, 938, 1,875, or 3,750 ppm for 8 days and 10 male and 10 female rats given 469, 938, or 1,875 ppm

for 3, 6, 12, or 18 months were designated for toxicokinetic studies. Toxicokinetic studies were also conducted on 10 male and 10 female randomly selected 3,750 ppm core study rats at 3, 6, 12, and 18 months. Groups of 50 male and 50 female mice were fed diets containing 0, 833, 2,500, or 7,500 ppm for 105 weeks. Additional groups of 36 male mice given 833, 2,500, or 7,500 ppm for 8 days and 10 male and 10 female mice given 833, 2,500, or 7,500 ppm for 12 months were designated for toxicokinetic studies.

Source and Specification of Animals

Male and female F344/N rats and $B63CF_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 2-year studies. Rats and mice were quarantined for 11 (males) or 12 (females) days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 6 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix M).

Animal Maintenance

Rats were housed three (males) or five (females) per cage and mice were housed one (males) or five (females) per cage. Feed and water were available *ad libitum*. Feed consumption was measured every 4 weeks. Cages were changed twice weekly or once weekly (male mice); racks were changed and 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

All animals were observed twice daily. Clinical findings were recorded every 4 weeks and at the end of the studies. Body weights were recorded at the beginning of the studies, on day 8, every 4 weeks, and at the end of the studies.

Toxicokinetics

Blood samples were collected from the retroorbital sinus of toxicokinetic study rats on day 8 and at 3, 6, 12, and 18 months, from 3,750 ppm core study rats at 3, 6, 12, and 18 months, and from toxicokinetic study mice on day 8 and at 12 months. Collection was made at 12 time points on day 8 and at 5 to 7 time points at 3, 6, 12, and 18 months. Blood was collected from two or three animals per group at each time point and from individual animals at two time points per collection period (8-day and 3-month rat bleeds) or at one time point per collection period (6-, 12-, and 18-month rat bleeds and 8-day and 12-month mouse bleeds). At 18 months, 13 to 14 previously undosed male and female rats and mice were given a single dose of 100 mg/kg (rats) or 200 mg/kg (mice) in 0.2% methylcellulose and 0.1% Tween 80 by gavage for toxicokinetic studies in aged animals. Blood was collected from two or three animals per gender at five time points, and each animal was bled once. The time points at which blood was collected from each group are listed in Table 1. Blood was collected in tubes containing potassium EDTA as an anticoagulant. The red cell fraction was separated from the plasma by centrifugation, and the plasma was stored at up to -20° C until analysis for anthraquinone concentration.

Pathology

Complete necropsies and microscopic examinations were performed on core study rats and mice. Interim evaluations of 0 and 3,750 ppm rats were conducted at 3 and 12 months; left and right kidneys and the liver were weighed at 12 months. 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 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. **For extended evaluation of renal tubule proliferative lesions in male mice, kidneys were step-sectioned a 1-mm intervals, and additional sections were obtained from each kidney.** Tissues examined microscopically are listed in Table 1.

At the 3-month interim evaluation necropsy, the right kidneys of male and female 0 and 3,750 ppm rats were bisected longitudinally, and each half was placed in a vial. Samples were processed and analyzed for soluble protein content and α2u-globulin concentration as described for the 14-week study. Parameters measured 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, spleen, thyroid gland, and urinary bladder of male and female rats, the liver, spleen, thyroid gland, and urinary bladder of male and female mice, pancreatic islets of male mice, and skin, stomach, and thymus of female mice.

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 Anthraquinone

14-Week Studies	2-Year Studies
Study Laboratory Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species Rats: F344/N Mice: B6C3F$_1$	Rats: F344/N Mice: B6C3F$_1$
Animal Source Taconic Farms (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies Rats: 11 (males) or 12 (females) days Mice: 13 (males) or 14 (females) days	11 (males) or 12 (females) days
Average Age When Studies Began 6 to 7 weeks	6 to 7 weeks
Date of First Exposure Rats: January 17 (males) or 18 (females), 1994 Mice: January 26 (males) or 27 (females), 1994	Rats: November 14 (males) or 15 (females), 1994 Mice: October 31 (males) or November 1 (females), 1994
Duration of Exposure 14 weeks	105 weeks
Date of Last Exposure Rats: April 18 (males) or 19 (females), 1994 Mice: April 27 (males) or 28 (females), 1994	Rats: November 11-12 (males) or 13-15 (females), 1996 Mice: October 28-30 (males) or October 30-November 1 (females), 1996
Necropsy Dates Rats: April 18 (males) or 19 (females), 1994 Mice: April 27 (males) or 28 (females), 1994	Rats: November 11-12 (males) or 13-15 (females), 1996 (core study) February 13 (males) or 14 (females), 1995 (3-month interim evaluation November 1, 1995 (12-month interim evaluation) Mice: October 28-30 (males) or October 30-November 1 (females), 1996
Average Age at Necropsy 20 weeks	3-month interim evaluation: 19 to 20 weeks 12-month interim evaluation: 56 to 57 weeks Terminal sacrifice: 110 (males) or 111 (females) weeks

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

14-Week Studies	2-Year Studies
Size of Study Groups 10 males and 10 females	Rats: 60 males and 60 females (0 and 3,750 ppm) 50 males and 50 females (469, 938, and 1,875 ppm) Mice: 50 males and 50 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 14-week studies
Animals per Cage Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 3 (males) or 5 (females) Mice: 1 (males) or 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo
Diet NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum* except during urine collection periods	Same as 14-week studies; available *ad libitum*
Water Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 14-week studies
Cages Polycarbonate (Lab Products, Inc., Maywood, NJ) changed twice weekly or once weekly (male mice)	Same as 14-week studies
Bedding Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ) changed twice weekly or once weekly (male mice)	Same as 14-week studies
Cage Filters Dupont 2024 spun-bonded polyester (Snow Filtration Co., Cincinnati, OH) changed every 2 weeks	Same as 14-week studies
Racks Stainless steel (Lab Products, Inc., Maywood, NJ) changed and rotated every 2 weeks	Same as 14-week studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Exposure Concentrations 0, 1,875, 3,750, 7,500, 15,000, or 30,000 ppm in feed, available *ad libitum* except during urine collection periods	Rats: 0, 469, 938, 1,875, or 3,750 ppm in feed, available *ad libitum* Mice: 0, 833, 2,500, or 7,500 ppm in feed, available *ad libitum*
Type and Frequency of Observation Observed twice daily; animals were weighed at study initiation, once weekly, and at the end of the studies. Clinical findings were recorded once weekly and at the end of the studies. Feed consumption was recorded twice weekly or once weekly (male mice).	Observed twice daily; animals were weighed at the beginning of the studies, on day 8, every 4 weeks, and at the end of the studies. Clinical findings were recorded every 4 weeks and at the end of the studies. Feed consumption was recorded every 4 weeks.

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

14-Week Studies	2-Year Studies
Method of Sacrifice Carbon dioxide asphyxiation	Same as 14-week studies
Necropsy Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lungs, right testis, and thymus. Soluble protein and α2u-globulin concentrations were measured in kidney homogenate.	Necropsies were performed on core study rats and mice. Soluble protein and α2u-globulin concentrations were measured in kidney homogenate at the 3-month interim evaluation. Organs weighed at the 12-month interim evaluation in rats were left and right kidneys and the liver.
Clinical Pathology Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 22 and from all core study rats and mice surviving to the end of the study for hematology and clinical chemistry (rats) determinations. All core and clinical pathology study rats were placed in metabolism cages for urine collection. Urine was collected from clinical pathology study rats on days 8 and 26 and from core study rats on day 89. ***Hematology:*** erythrocyte, reticulocyte, and platelet counts; hematocrit values; hemoglobin concentration; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile salts ***Urinalysis:*** creatinine, glucose, total protein, aspartate aminotransferase, *N*-acetyl-β-D-glucosaminidase, γ-glutamyltransferase, total volume, and specific gravity	None
Histopathology Complete histopathology was performed on all core study 0 and 30,000 ppm rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice only), heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland (with adjacent skin), 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. In addition, the bone marrow, liver, kidney, spleen, and thyroid gland of male and female rats, the urinary bladder of female rats, and the liver and urinary bladder of mice were examined in all lower exposure groups.	Complete histopathology was performed on all core study rats and all mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice only), heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland (with adjacent skin) (except male mice), nose, ovary, pancreas, pancreatic islets, 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.

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

14-Week Studies	2-Year Studies
Sperm Motility and Vaginal Cytology At the end of the studies, sperm samples were collected from all core study male rats and mice in the 0, 7,500, 15,000, and 30,000 ppm groups for sperm count and motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda epididymis, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from core study 0, 7,500, 15,000, and 30,000 ppm female rats and mice for vaginal cytology evaluations. The following parameters were evaluated: estrous cycle length and relative frequency of the estrous stages.	None
Toxicokinetic Studies None	Blood was collected from the retroorbital sinus of 18 male rats given 469, 938, 1,875 or 3,750 ppm and 36 male mice given 833, 2,500, or 7,500 ppm anthraquinone for 8 days. Blood was collected from the retroorbital sinus of 10 male and 10 female rats given 469, 938, 1,875 or 3,750 ppm and 10 male and 10 female mice given 833, 2,500, or 7,500 ppm anthraquinone; blood was collected from rats at 3, 6, 12, and 18 months and from mice at 12 months. Blood was collected at the following times: **Rats** 8 days 0800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 0200, 0400, and 0600 3 months 0800, 1130, 1400, 1730, 2100, 0030, and 0400 6 months 0600, 1100, 1600, 2100, and 0200 12 months 0600, 1100, 1600, 2100, and 0200 18 months 0600, 1100, 1600, 2100, and 0200 **Mice** 8 days 0800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 0200, 0400, and 0600 12 months 0600, 1100, 1600, 2100, and 0200
Single-Dose Toxicokinetics in Aged Animals None	Blood was collected from the retroorbital sinus of 14 male and 14 female rats and 14 male and 13 female mice after a single gavage dose of 100 mg/kg (rats) or 200 mg/kg (mice) in 0.2% methylcellulose and 0.1% Tween 80 for determination of anthraquinone concentrations in plasma. Blood was collected at the following time points after dosing: Rats: 2, 6, 12, 24, and 36 hours Mice: 1, 2, 4, 8, and 12 hours

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 or missing 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 D5 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 is represented as 1−P with the letter N added (e.g., P=0.99 is presented as P=0.01N). For

neoplasms and nonneoplastic lesions detected at the interim evaluations, 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). Soluble protein and α2u-globulin concentrations were analyzed by Dunnett's one-tailed *t*-test for comparing means to the 0 ppm group and Duncan's multiple range test for comparing all means to each other. Hematology, clinical chemistry, urinalysis, toxicokinetic, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. 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 anthraquinone was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, micronucleated erythrocytes in mouse 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 have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

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

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

14-WEEK STUDY

All rats survived until the end of the study (Table 2). Exposed groups of males did not differ significantly from controls in final mean body weight or body weight gain. Final mean body weights and body weight gains of exposed groups of females were significantly less than those of the control group. Feed consumption by exposed male rats was lower than that by the controls during the first week of the study. Feed consumption by the 1,875 and 3,750 ppm males was comparable to that by the controls by the end of the study, while feed consumption by males exposed to 7,500 ppm or greater was increased. Although feed consumption by exposed female groups was less that by the controls at the beginning of the study, it was greater than that by the controls at the end of the study. The increased feed consumption may have been due to rats digging in the feeders and possibly scattering feed, an indication of poor palatability; the reduced feed consumption during week one could have been due to the poor palatability of the feed. Dietary concentrations of 1,875, 3,750, 7,500, 15,000, and 30,000 ppm anthraquinone resulted in average daily doses of approximately 135, 275, 555, 1,130, and 2,350 mg anthraquinone/kg body weight to males and females. There were no exposure-related clinical findings.

TABLE 2
Survival, Body Weights, and Feed Consumption of Rats in the 14-Week Feed Study of Anthraquinone

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Feed Consumption[c] Week 1	Week 14
Male							
0	10/10	122 ± 2	327 ± 4	205 ± 4		15.0	15.8
1,875	10/10	123 ± 2	342 ± 3	219 ± 3	104	14.7	16.0
3,750	10/10	122 ± 2	345 ± 5	222 ± 6	105	13.9	15.9
7,500	10/10	120 ± 2	327 ± 6	207 ± 5	99	12.6	16.7
15,000	10/10	122 ± 2	334 ± 5	212 ± 5	102	12.9	16.8
30,000	10/10	123 ± 2	316 ± 5	193 ± 5	96	12.2	16.9
Female							
0	10/10	106 ± 1	205 ± 2	99 ± 2		10.9	8.2
1,875	10/10	106 ± 2	196 ± 3*	90 ± 3**	96	9.4	9.8
3,750	10/10	106 ± 2	186 ± 3**	80 ± 2**	91	8.6	9.8
7,500	10/10	105 ± 2	179 ± 2**	74 ± 2**	87	7.3	9.7
15,000	10/10	107 ± 2	181 ± 3**	74 ± 3**	88	6.1	10.1
30,000	10/10	107 ± 1	172 ± 1**	65 ± 2**	84	6.0	10.2

* 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. Differences from the control group are not significant by Williams' or Dunnett's test.
[c] Feed consumption is expressed as grams per animal per day

On day 4, there was evidence of a minimal chemical-related erythrocytosis, demonstrated by increased hematocrit values, hemoglobin concentrations, and/or erythrocyte counts in 3,750 ppm or greater males and 7,500 ppm or greater females (Table F1). The erythrocytosis was transient and was replaced by minimal anemia by the third week of the study (demonstrated by generally decreased hematocrit values, hemoglobin concentrations, and erythrocyte counts). The anemia persisted and involved all exposed groups of males and females at the end of the study. There was evidence of an erythropoietic response to the anemia, demonstrated by reticulocyte count increases in exposed groups of females on day 22 and all exposed groups at the end of the study. Accompanying the increased reticulocyte counts were minimal increases in the mean cell volumes. On day 22 and at week 14, mean cell hemoglobin concentrations decreased minimally in 30,000 ppm males and all exposed groups of females, and minimal to mild exposure-related increases in platelet counts were observed in all exposed groups.

There was evidence of a hepatocellular response to anthraquinone exposure demonstrated by increased serum alanine aminotransferase and sorbitol dehydrogenase activities and bile salt concentrations (Table F1). On day 4, alanine aminotransferase activities were minimally increased in 7,500 ppm or greater males and 3,750 ppm or greater females; sorbitol dehydrogenase activities were mildly to moderately increased in all exposed groups. Also on day 4, bile salt concentrations were mildly increased in all exposed groups of males and 3,750 ppm or greater females. With time, these effects ameliorated. For females, bile salt and alanine aminotransferase effects were transient, and in fact, alanine aminotransferase activities were minimally decreased in most exposed groups on day 22 and at week 14; sorbitol dehydrogenase activities remained mildly increased, although not consistently among exposed groups, throughout the study. For males, the bile salt and alanine aminotransferase effects noted on day 4 disappeared and were replaced by decreases in alanine aminotransferase activities and/or bile salt concentrations by week 14. At week 14, however, two 30,000 ppm males had exceptionally high values for sorbitol dehydrogenase, alanine aminotransferase, and bile salts (data not shown). Thus, despite the lack of statistical significance for the group means, increases for the affected animals would be consistent with a hepatic response. In an apparent incongruous response, serum alkaline phosphatase activity (a marker of cholestasis) was mildly to moderately decreased in 7,500 and 15,000 ppm females on day 4 and all exposed groups of rats on day 22 and at week 14.

Creatinine concentration, a marker of renal function, was minimally increased in all groups of exposed males at all time points; females also demonstrated this effect but with less consistency (Table F1). Urea nitrogen concentration, another marker of renal function, also demonstrated minimal increases in exposed males on day 22 and at week 14; females were unaffected. On day 22 and at week 14, total protein and albumin concentrations were increased in all exposed groups of rats.

A transient exposure concentration-related decrease in urine volume and increases in urine specific gravity and urine creatinine concentration occurred in exposed males on day 8; these effects resolved by day 26,

and exposed females were unaffected (Table F1). For males and females, an effect on kidney function was demonstrated by increases in urine protein and glucose concentrations and aspartate aminotransferase and *N*-acetyl-β-D-glucosaminidase activities. On day 8, normalized urine aspartate aminotransferase activities were increased in all groups of exposed females. On day 26 and at week 13, normalized urine protein, glucose concentrations, and aspartate aminotransferase and *N*-acetyl-β-D-glucosaminidase activities were increased in all groups of exposed males. In exposed females at week 13, normalized urine aspartate aminotransferase and *N*-acetyl-β-D-glucosaminidase activities increased, as did urine protein concentrations in 15,000 and 30,000 ppm females.

Liver and kidney weights of exposed groups of males and females were significantly greater than those of the controls (Table G1). Absolute testis weights were significantly greater in all exposed groups of males, as were relative testis weights in males exposed to 7,500 ppm or greater.

Epididymal spermatozoal measurements of exposed males did not differ significantly from those of the controls (Table H1). Estrous cycles of 15,000 and 30,000 ppm females were longer than that of the control group (Table H2).

Histologic lesions associated with exposure to anthraquinone were present in the liver, kidney, spleen, bone marrow, and thyroid gland of males and females and in the urinary bladder of females (Table 3). All exposed rats had liver hypertrophy. Liver hypertrophy was characterized by swollen, centrilobular hepatocytes containing increased quantities of eosinophilic cytoplasm with normal basophilic stippling more prominent at the periphery of the cell and less dense in the center. At lower exposure concentrations, hypertrophied hepatocytes were immediately adjacent to the central vein. At higher exposure concentrations, the hypertrophied hepatocytes occupied a larger area of the lobule surrounding the central vein. A no-effect level was not achieved for this lesion. The severity was minimal at 1,875 ppm, increased to mild at 3,750 ppm or greater in males and females, and was moderate in 30,000 ppm males.

TABLE 3
Incidences of Selected Nonneoplastic Lesions in Rats in the 14-Week Feed Study of Anthraquinone

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
Male						
Liver[a]	10	10	10	10	10	10
Hypertrophy[b]	0	10** (1.0)[c]	10** (1.8)	10** (2.0)	10** (2.0)	10** (2.9)
Kidney	10	10	10	10	10	10
Hyaline Droplet Accumulation	0	10** (2.0)	10** (2.0)	10** (2.2)	10** (2.0)	10** (2.8)
Nephropathy	10 (1.0)	10 (1.7)	10 (1.6)	10 (1.7)	10 (2.0)	10 (2.2)
Spleen	10	10	10	10	10	10
Congestion	0	10** (2.0)	10** (2.0)	10** (2.0)	10** (2.0)	10** (2.0)
Hematopoietic Cell Proliferation	0	10** (1.0)	9** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Pigmentation	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Bone Marrow	10	10	10	10	10	10
Hyperplasia	0	3 (1.0)	5* (1.0)	8** (1.0)	6** (1.2)	5* (1.0)
Thyroid Gland	10	10	10	10	10	10
Follicular Cell, Hypertrophy	0	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Female						
Liver	9	10	10	10	10	10
Hypertrophy	0	10** (1.0)	10** (2.0)	10** (1.8)	10** (2.0)	10** (2.0)
Kidney	10	10	10	10	10	10
Hyaline Droplet Accumulation	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.2)	10** (2.0)
Nephropathy	3 (1.0)	2 (1.0)	3 (1.0)	5 (1.0)	8* (1.0)	10** (1.7)
Spleen	9	10	10	10	10	10
Congestion	0	10** (1.1)	10** (1.2)	10** (1.5)	10** (1.3)	10** (1.0)
Hematopoietic Cell Proliferation	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Pigmentation	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Bone Marrow	10	10	10	10	10	10
Hyperplasia	0	7** (1.0)	7** (1.0)	10** (1.0)	9** (1.0)	10** (2.0)
Thyroid Gland	10	10	10	10	10	10
Follicular Cell, Hypertrophy	0	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Urinary Bladder	9	10	10	10	9	10
Inflammation	0	0	0	0	1 (1.0)	6** (1.2)
Transitional Epithelium, Hyperplasia	0	0	0	0	0	9** (1.9)

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

Variably sized eosinophilic hyaline droplets occurred within the cytoplasm of renal tubule epithelial cells and tubule lumens of all exposed males and females (Table 3). The severities of the lesions ranged from mild to moderate in males and minimal to mild in females. In females, the droplets were somewhat smaller, and there were fewer angular or crystalline-appearing droplets present than in males. In sections stained with hematoxylin and eosin, the droplets were brightly eosinophilic in males, whereas in females, the droplets appeared yellow-brown to dull eosinophilic. In sections stained with Mallory-Heidenhain, droplets in male kidneys were red while those in female kidneys appeared brown-red. Droplets in females were PAS positive, but those in males were not. Droplets from males and females were negative for iron and acid fast stains. Nephropathy was present in all male rats including the controls, but the lesion was more severe in all exposed groups of males and in 30,000 ppm females than in the controls. The incidences of nephropathy in 15,000 and 30,000 ppm females were significantly greater than that in the controls.

The amount of α2u-globulin was quantitated in homogenates of kidneys from male rats at the end of the study using an enzyme-linked immunoassay (ELISA). Figure 1 shows that the concentrations of α2u-globulin in the kidneys were significantly greater in all exposed groups of males than in the control group.

Congestion, hematopoietic cell proliferation (except one 3,750 ppm male), and pigmentation of the spleen occurred in all exposed rats (Table 3). Sections of the spleens taken from exposed rats and stained with Perl's iron stain appeared to contain more iron pigment than sections from controls. Hematopoietic cell proliferation was characterized by an increase in the number and size of foci in the red pulp. The incidences of bone marrow hyperplasia in all exposed groups except 1,875 ppm males were significantly greater than those in the controls; however, the lesion was generally of minimal severity.

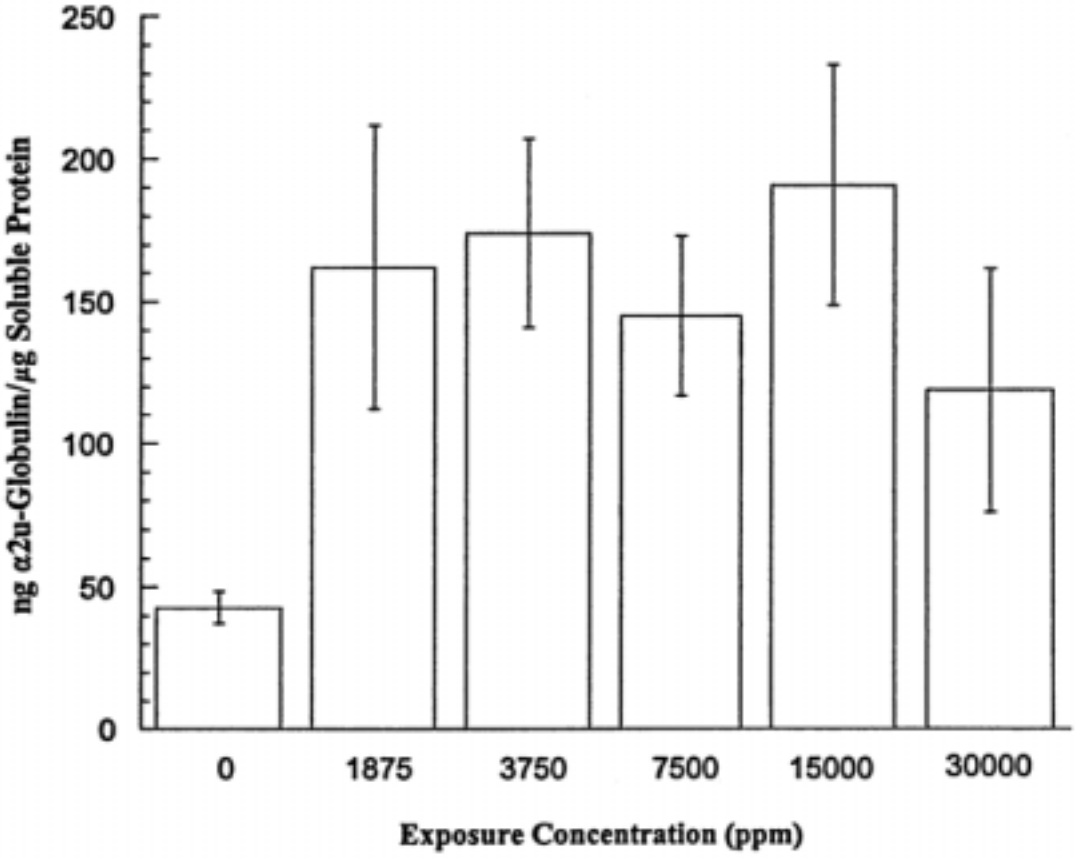


FIGURE 1
Concentrations of α2u-Globulin (ng/μg Soluble Protein) in the Supernatant of Kidney Homogenates from Male Rats in the 14-Week Feed Study of Anthraquinone

Thyroid gland follicular cell hypertrophy was present in all males and females exposed to 3,750 ppm or greater (Table 3). This was a minimal lesion characterized by a slight enlargement of the thyroid gland epithelium and an increase in the amount of cytoplasm in the apical portion of follicular cells. The cytoplasm of affected cells also appeared slightly more vacuolated than that in the controls.

Incidences of inflammation and transitional epithelial hyperplasia of the mucosa in the urinary bladder of 30,000 ppm females were significantly greater than those in the controls (Table 3). The lesions consisted of a thickening of the epithelium, a few mononuclear cells within the lamina propria, and a mixture of mononuclear cells and a few polymorphonuclear cells in the transitional epithelium. These lesions were not observed in male rats.

Exposure Concentration Selection Rationale: Toxic responses in the kidney and liver served as the basis for selection of exposure concentrations for the 2-year rat study. Kidney weights were significantly increased in all exposed groups. Although nephropathy was present in control males, the severities were greater in all exposed groups. In females, the incidences of nephropathy were significantly increased at 15,000 and 30,000 ppm. Significantly increased liver weights and significant increases in the incidences of hepatocellular hypertrophy occurred in all exposed groups. The severity of hepatocellular hypertrophy increased from minimal at 1,875 ppm to mild at 3,750 ppm. Based on the increased severity of nephropathy and hepatocellular hypertrophy, 1,875 ppm was considered an adequate high exposure concentration for the 2-year study, and 469 and 938 ppm were selected for the lower concentrations.

The role of centrilobular hypertrophy in rat hepatocarcinogenesis is not well characterized in long-term studies, and its impact on survival is not known. **Hypertrophy is not a proliferative lesion, and its potential to progress to frank toxicity with continued chemical exposure has not been extensively evaluated. Therefore, the 2-year rat study design included groups exposed to 3,750 ppm anthraquinone with 3- and 12-month interim evaluations to monitor the development of hepatotoxicity. This exposure concentration was selected because it was the lowest that produced**

centrilobular hypertrophy of mild severity in the 14-week study. Although mild centrilobular hypertrophy was also observed at 7,500 and 15,000 ppm, the use of these higher concentrations would have increased the risk for development of more severe nephropathy, which could have resulted in early mortality.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 4 and in the Kaplan-Meier survival curves (Figure 2). Survival of all exposed groups of male rats was similar to that of the controls. Survival of all exposed groups of female rats was significantly greater than that of the controls.

TABLE 4
Survival of Rats in the 2-Year Feed Study of Anthraquinone

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Male					
Animals initially in study	60	50	50	50	60
3-Month interim evaluation[a]	5				5
12-Month interim evaluation[a]	5				5
Moribund	21	21	16	14	17
Natural deaths	7	6	12	10	11
Animals surviving to study termination	22[e]	23	22	26	22
Percent probability of survival at end of study[b]	44	46	44	52	44
Mean survival (days)[c]	674	692	684	690	665
Survival analysis[d]	P=1.000	P=0.639	P=0.823N	P=0.324N	P=1.000N
Female					
Animals initially in study	60	50	50	50	60
3-Month interim evaluation[a]	5				5
12-Month interim evaluation[a]	5				5
Moribund	14	7	12	7	6
Natural deaths	13	3	3	6	4
Animals surviving to study termination	23	40	35	37	40
Percent probability of survival at end of study	46	80	70	74	80
Mean survival (days)	691	709	693	713	718
Survival analysis	P=0.008N	P=0.002N	P=0.029N	P=0.006N	P<0.001N

[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 vehicle controls are in the exposed columns. A negative trend or lower mortality in an exposed group is indicated by **N**.
[e] Includes one animal that died during the last week of the study

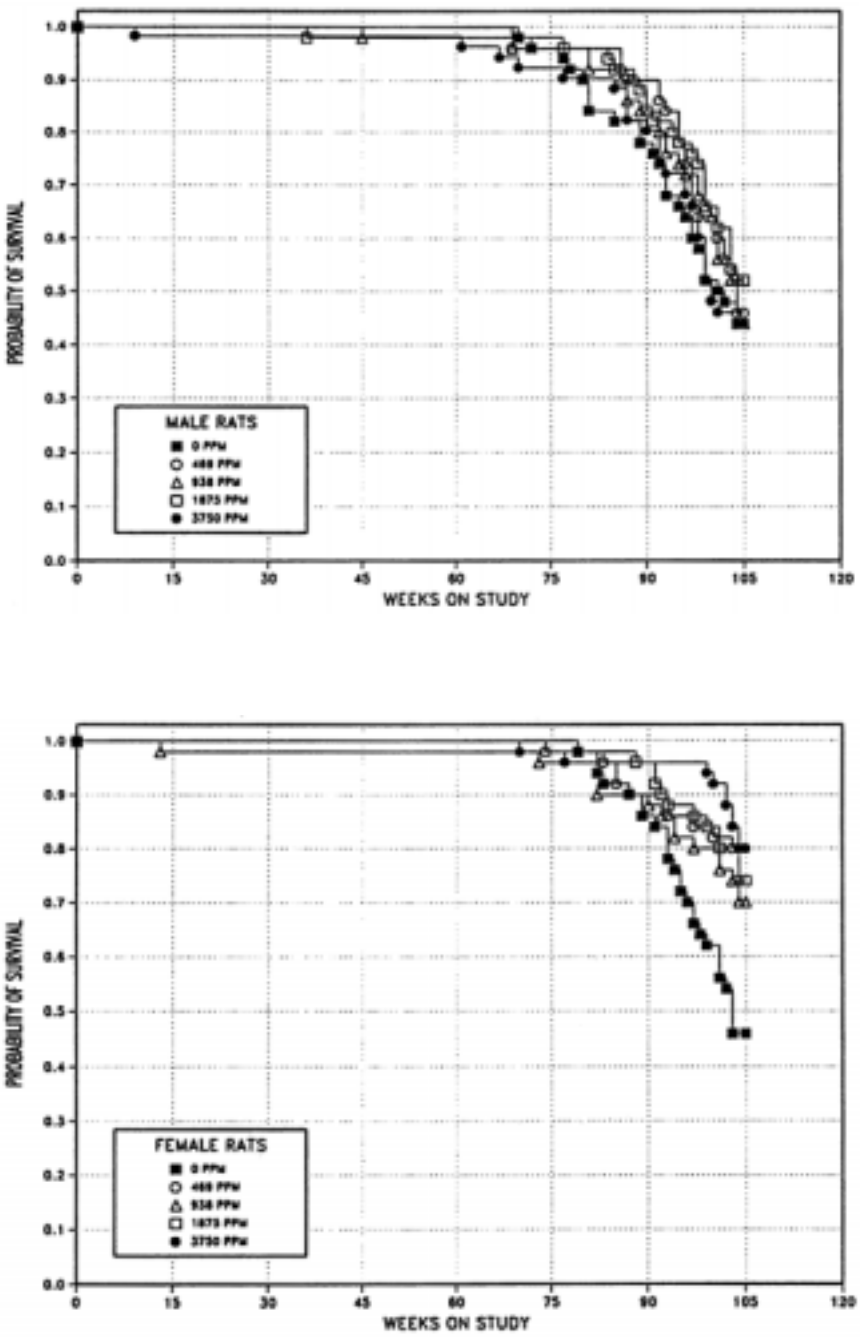
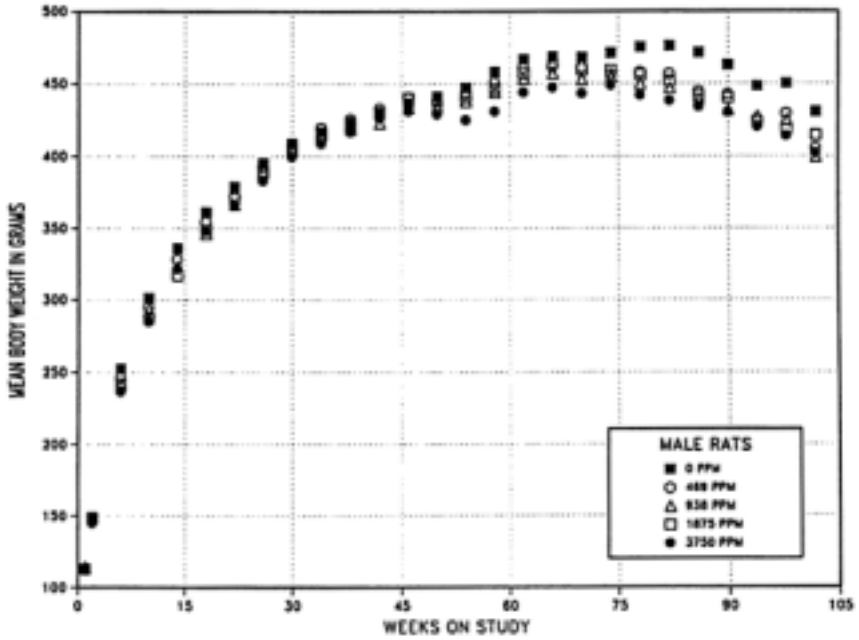


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Anthraquinone in Feed for 2 Years

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of exposed groups of males were less than those of the control group during the latter part of the study (Figure 3 and Table 5). Mean body weights of exposed females were less than those of the controls throughout most of the study (Figure 3 and Table 6). Feed consumption by all groups of males and females was similar to that by the control groups (Tables K1 and K2). Dietary concentrations of 469, 938, 1,875, and 3,750 ppm anthraquinone delivered average daily doses of approximately 20, 45, 90, and 180 mg anthraquinone/kg body weight to males and 25, 50, 100, and 200 mg/kg to females. There were no clinical findings that could be attributed to anthraquinone exposure.



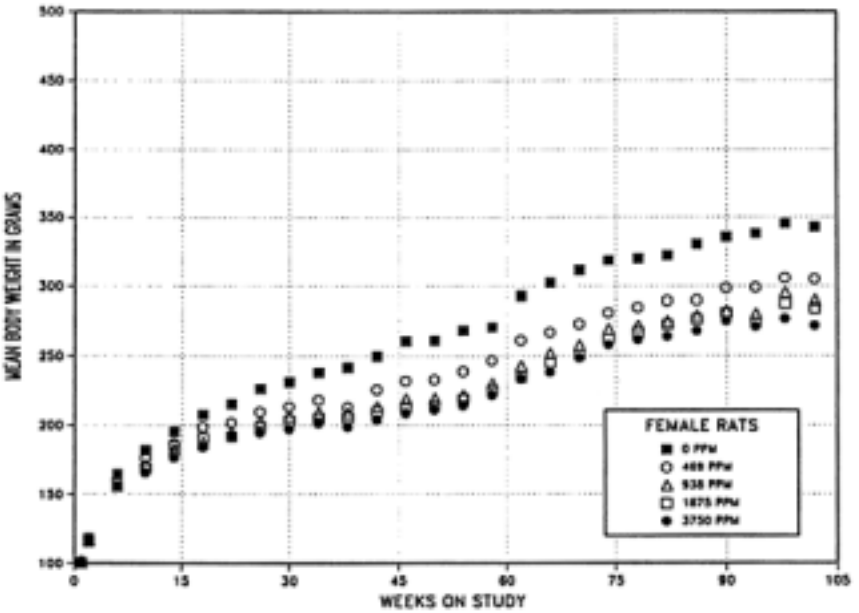


FIGURE 3
Growth Curves for Male and Female Rats Exposed to Anthraquinone in Feed for 2 Years

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Anthraquinone

Weeks on Study	0 ppm		469 ppm			938 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
1	113	60	112	99	50	114	101	50
2	149	60	148	99	50	149	100	50
6	252	60	246	98	50	241	96	50
10	301	60	294	98	50	293	97	50
14	336	60	329	98	50	323	96	50
18[a]	361	55	354	98	50	346	96	50
22	379	55	373	99	50	367	97	50
26	395	55	393	99	50	389	99	50
30	409	55	407	100	50	404	99	50
34	416	55	419	101	50	414	99	50
38	423	55	425	101	50	418	99	50
42	430	55	432	101	50	423	98	50
46	436	55	440	101	50	433	99	49
50	441	55	439	100	50	434	98	49
54[a]	447	50	443	99	50	438	98	49
58	458	50	450	98	50	444	97	49
62	466	50	461	99	50	453	97	49
66	468	50	461	99	50	457	98	49
70	468	50	461	99	48	453	97	48
74	471	48	456	97	48	455	97	48
78	475	46	458	96	48	449	95	48
82	476	42	456	96	48	447	94	46
86	471	41	444	94	48	439	93	45
90	462	39	442	96	45	432	93	42
94	448	34	425	95	42	427	95	38
98	450	30	429	95	33	425	94	35
102	430	25	405	94	29	400	93	28
Mean for weeks								
1-13	204		200	98		199	98	
14-52	403		401	100		395	98	
53-102	461		445	97		440	95	

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Anthraquinone

Weeks on Study	1,875 ppm			3,750 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	113	100	50	113	100	60
2	148	99	50	145	97	60
6	242	96	50	237	94	60
10	286	95	50	285	95	59
14	317	94	50	322	96	59
18[a]	349	97	50	348	96	54
22	370	98	50	366	97	54
26	390	99	50	383	97	54
30	405	99	50	400	98	54
34	414	100	50	409	98	54
38	420	99	49	417	99	54
42	429	100	49	426	99	54
46	440	101	49	431	99	54
50	437	99	49	429	97	54
54[a]	440	99	49	425	95	49
58	446	98	49	430	94	49
62	457	98	49	444	95	48
66	464	99	49	447	95	48
70	459	98	49	443	95	47
74	459	98	49	449	95	46
78	456	96	48	442	93	45
82	452	95	48	438	92	45
86	441	94	46	434	92	44
90	440	95	44	430	93	40
94	423	94	41	420	94	36
98	418	93	38	414	92	32
102	414	96	31	402	93	23
Mean for weeks						
1-13	197	97		195	96	
14-52	397	99		393	98	
53-102	444	96		432	94	

[a] Interim evaluations occurred during weeks 14 and 51 for the 0 and 3,750 ppm groups.

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

Weeks on Study	0 ppm		469 ppm			938 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
1	101	60	101	101	50	102	101	50
2	118	60	119	100	50	117	99	50
6	165	60	161	98	50	156	95	50
10	182	60	176	97	50	171	94	50
14	195	60	187	96	50	182	93	49
18[a]	208	55	199	96	50	187	90	49
22	215	55	202	94	50	195	91	49
26	226	55	210	93	50	203	90	49
30	231	55	213	92	50	206	89	49
34	238	55	218	92	50	209	88	49
38	242	55	213	88	50	209	86	49
42	250	55	226	90	50	214	86	49
46	261	55	232	89	50	219	84	49
50	261	55	233	89	50	219	84	49
54[a]	269	50	239	89	50	222	83	49
58	271	50	247	91	50	230	85	49
62	293	50	261	89	50	243	83	49
66	302	50	267	88	50	252	83	49
70	311	50	273	88	50	258	83	49
74	318	50	281	88	50	270	85	48
78	320	50	285	89	49	272	85	48
82	322	49	290	90	49	275	85	48
86	330	46	290	88	46	280	85	45
90	335	43	299	89	45	283	84	45
94	338	39	299	89	43	281	83	43
98	345	33	306	89	42	296	86	40
102	343	28	305	89	41	290	85	38
Mean for weeks								
1-13	142		139	98		137	96	
14-52	233		213	91		204	88	
53-102	315		280	89		266	84	

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

Weeks on Study	1,875 ppm			3,750 ppm		
	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	100	100	50	102	101	60
2	116	98	50	117	99	60
6	156	95	50	157	95	60
10	169	93	50	166	91	60
14	185	95	50	177	90	60
18[a]	191	92	50	184	89	55
22	192	89	50	191	89	55
26	199	88	50	195	86	55
30	203	88	50	197	85	55
34	205	86	50	202	85	55
38	205	85	50	199	82	55
42	210	84	50	204	82	55
46	213	82	50	209	80	55
50	214	82	50	211	81	55
54[a]	219	82	50	214	80	50
58	225	83	50	222	82	50
62	235	80	50	234	80	50
66	245	81	50	238	79	50
70	251	80	50	249	80	50
74	262	82	50	258	81	49
78	267	84	50	262	82	48
82	273	85	49	264	82	48
86	277	84	49	268	81	48
90	281	84	48	275	82	48
94	274	81	44	271	80	48
98	288	83	43	277	80	48
102	284	83	40	272	79	46
Mean for weeks						
1-13	135	95		136	96	
14-52	202	87		197	85	
53-102	260	83		254	81	

[a] Interim evaluations occurred during weeks 14 and 51 for the 0 and 3,750 ppm groups.

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 kidney (with secondary lesions in the parathyroid gland, bone, forestomach, glandular stomach, and lung), urinary bladder, liver, skin, thyroid gland, 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.

Kidney: The incidences of hyaline droplet accumulation in 3,750 ppm males and females at 3 months, 3,750 ppm males at 12 months, and in all exposed groups except 938 ppm males at 2 years were significantly greater than those in the controls (Tables 7, A5, and B5). In males at 3 months, hyaline droplets were spherical, angular, or crystalline bright eosinophilic droplets contained within the cytoplasm of proximal renal tubule cells or within the tubule lumen; in females, the droplets were less prominent, and angular and/or crystalline forms were not evident. At 12 months, hyaline droplets in 3,750 ppm males and females were less prominent and appeared less eosinophilic in males than those observed at 3 months. At 2 years, hyaline droplets were smaller and less eosinophilic than those observed earlier and frequently exhibited a change in tinctorial quality to rust or orange brown. In addition, angular and crystalline forms were not evident at 2 years.

Incidences of nephropathy in all groups of exposed females were significantly greater than that in the controls at 2 years (Tables 7 and B5). The incidences of nephropathy in females at 3 and 12 months were slightly increased. Nephropathy was present in all male rats including the controls at 3 and 12 months and at 2 years; however, severities in exposed groups were increased relative to the control group (Tables 7 and A5). Associated with nephropathy in male rats at 2 years were exposure concentration-related increased

TABLE 7
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney and Urinary Bladder in Rats in the 2-Year Feed Study of Anthraquinone

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Male					
3-Month Interim Evaluation					
Kidney[a]	5				5
Accumulation, Hyaline Droplet[b]	0				5** (3.0)[c]
Nephropathy	5 (1.2)				5 (1.6)
12-Month Interim Evaluation					
Kidney	5				5
Accumulation, Hyaline Droplet	0				5** (2.8)
Nephropathy	5 (1.0)				5 (1.8)
Medulla, Mineralization	0				5** (1.2)
2-Year Study					
Kidney	50	50	50	50	50
Accumulation, Hyaline Droplet	3 (1.0)	14** (1.2)	10 (1.2)	16** (1.1)	16** (1.1)
Nephropathy	50 (2.2)	50 (3.1)	50 (3.1)	50 (3.0)	50 (3.0)
Pigmentation	25 (1.5)	31 (1.1)	36* (1.1)	38** (1.1)	33* (1.0)
Medulla, Mineralization	30 (1.0)	42** (1.0)	46** (1.0)	47** (1.2)	49** (1.6)
Renal Tubule, Hyperplasia	3 (1.0)	7 (1.4)	3 (1.3)	9 (1.6)	9 (1.8)
Transitional Epithelium, Hyperplasia	28 (1.1)	45** (1.2)	44** (1.4)	48** (1.4)	48** (1.4)
Renal Tubule, Adenoma[d]					
Overall rate[e]	1/50 (2%)	3/50 (6%)	9/50 (18%)	5/50 (10%)	3/50 (6%)
Adjusted rate[f]	2.5%	6.8%	20.7%	11.4%	7.3%
Terminal rate[g]	1/22 (5%)	1/23 (4%)	3/22 (14%)	3/26 (12%)	1/22 (5%)
First incidence (days)	729 (T)	669	648	690	641
Poly-3 test[h]	P=0.474	P=0.333	P=0.010	P=0.119	P=0.308
Transitional Epithelium, Papilloma[i]	0	0	2	0	1
Urinary Bladder					
Transitional Epithelium, Papilloma[j]					
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	7/50 (14%)	3/49 (6%)
Adjusted rate	0.0%	2.3%	7.0%	15.5%	7.6%
Terminal rate	0/22 (0%)	1/23 (4%)	3/22 (14%)	3/26 (12%)	3/22 (14%)
First incidence (days)	—[k]	729 (T)	729 (T)	537	729 (T)
Poly-3 test	P=0.053	P=0.514	P=0.127	P=0.011	P=0.113
Female					
3-Month Interim Evaluation					
Kidney	5				5
Accumulation, Hyaline Droplet	0				5** (2.6)
Nephropathy	1 (1.0)				2 (1.0)
12-Month Interim Evaluation					
Kidney	5				5
Accumulation, Hyaline Droplet	2 (1.0)				5 (3.4)
Nephropathy	3 (1.0)				5 (1.0)
Medulla, Mineralization	1 (1.0)				4 (1.0)

TABLE 7
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney and Urinary Bladder in Rats in the 2-Year Feed Study of Anthraquinone

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Female (continued)					
2-Year Study					
Kidney	50	50	50	50	49
Accumulation, Hyaline Droplet	33 (1.1)	48** (1.2)	45** (1.4)	44** (1.7)	44** (1.7)
Nephropathy	39 (1.2)	49** (1.4)	47* (1.4)	49** (1.3)	49** (1.5)
Pigmentation	27 (1.2)	50** (1.1)	48** (1.1)	50** (1.0)	47** (1.1)
Medulla, Mineralization	17 (1.0)	25 (1.0)	27* (1.0)	28* (1.0)	20 (1.1)
Renal Tubule, Hyperplasia	0	12** (1.4)	13** (1.5)	15** (2.0)	11** (1.5)
Transitional Epithelium, Hyperplasia	0	5* (1.0)	12** (1.1)	3 (1.7)	10** (1.0)
Renal Tubule, Adenoma[l]	0	4	9**	7*	12**
Renal Tubule, Carcinoma	0	2	0	1	2
Renal Tubule, Adenoma or Carcinoma[m]					
Overall rate	0/50 (0%)	6/50 (12%)	9/50 (18%)	8/50 (16%)	14/49 (29%)
Adjusted rate	0.0%	12.9%	19.8%	16.7%	29.5%
Terminal rate	0/23 (0%)	6/40 (15%)	8/35 (23%)	5/37 (14%)	11/40 (28%)
First incidence (days)	—	730 (T)	570	611	689
Poly-3 test	P<0.001	P=0.020	P=0.002	P=0.006	P<0.001
Urinary Bladder	49	49	49	50	49
Transitional Epithelium, Hyperplasia	0	1 (1.0)	1 (2.0)	4 (2.8)	4 (1.5)
Transitional Epithelium, Papilloma	0	0	0	1	1
Transitional Epithelium, Carcinoma	0	0	0	0	1
Transitional Epithelium, Papilloma or Carcinoma[j]					
Overall rate	0/49 (0%)	0/49 (0%)	0/49 (0%)	1/50 (2%)	2/49 (4%)
Adjusted rate	0.0%	0.0%	0.0%	2.1%	4.2%
Terminal rate	0/23 (0%)	0/40 (0%)	0/35 (0%)	1/37 (3%)	2/40 (5%)
First incidence (days)	—	—	—	730 (T)	730 (T)
Poly-3 test	P=0.037	—[n]	—	P=0.522	P=0.264

* Significantly different (P≤0.05) from the control group by the Fisher exact test (interim evaluations) or the Poly-3 test (2-year study)
** P≤0.01
(T)Terminal sacrifice
[a] Number of animals with organ microscopically examined
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year feed studies with untreated control groups (mean ± standard deviation): 7/902 (0.8% ± 1.2%); range, 0%-4%
[e] Number of animals with neoplasm per number of animals with organ examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidences 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.
[i] Historical incidence: 1/902 (0.1% ± 0.5%); range, 0%-2%
[j] Historical incidence: 2/891 (0.2% ± 0.7%); range, 0%-2%
[k] Not applicable; no neoplasms in animal group
[l] Historical incidence: 0/901
[m] Historical incidence: 1/901 (0.1% ± 0.5%); range, 0%-2%
[n] Value of statistic cannot be computed.

incidences of parathyroid gland hyperplasia (0 ppm, 5/49; 469 ppm, 13/48; 938 ppm, 19/48; 1,875 ppm, 20/50; 3,750 ppm, 12/45), fibrous osteodystrophy (2/50, 4/50, 8/50, 11/50, 9/50), and mineralization of the forestomach (0/50, 0/50, 0/50, 2/50, 4/50), glandular stomach (2/50, 4/50, 7/50, 10/50, 10/50), and lung (1/50, 2/50, 3/50, 7/50, 6/50) (Table A5). These parathyroid gland, bone, stomach, and lung lesions are a consequence of perturbations in calcium homeostasis commonly seen in rats with marked nephropathy. The impaired renal function leads to secondary hyperparathyroidism.

At 2 years, the incidences of pigmentation in males exposed to 938 ppm or greater and in all exposed groups of females were significantly increased, as were the incidences of mineralization of the renal medulla in 3,750 ppm males at 12 months and in all exposed groups of males and in 938 and 1,875 ppm females at 2 years (Tables 7, A5, and B5).

At 2 years, the incidences of renal tubule hyperplasia (Plate 1) in all exposed groups of females were significantly greater than that in the controls; the incidences in most exposed groups of males were increased but not significantly (Tables 7, A5, and B5). There were positive trends in the incidences of renal tubule adenoma and of renal tubule adenoma or carcinoma (combined) in females, and the incidences of adenoma or carcinoma (combined) in all groups of exposed females were significantly increased and exceeded the historical control ranges (Tables 7, B3, and B4a). Renal tubule carcinomas (Plate 2) were present in two 469 ppm females, one 1,875 ppm female, and two 3,750 ppm females. The incidence of renal tubule adenoma in 938 ppm males was significantly greater than that in the controls, and the incidences in all exposed groups of males exceeded the historical control range (Tables 7, A3, and A4a); no renal tubule carcinomas were observed in male rats. Renal tubule hyperplasias were focal lesions characterized by increased numbers of tubular epithelial cells forming multiple layers that partially or totally filled the tubular lumen and usually caused slight dilation of the tubule. Renal tubule adenomas were larger than hyperplasias (usually five or more tubule diameters) with more complex structure and disruption of the tubule basement membrane. Larger adenomas often compressed the adjacent parenchyma. Carcinomas were differentiated

from adenomas by increased size, presence of hemorrhage, necrosis or locally invasive growth, and cellular anaplasia or atypia.

Many of the renal neoplasms, especially those in females, occurred in the absence of significant nephropathy, which suggests a primary response to anthraquinone. Also, many were morphologically different from spontaneous renal tubule neoplasms typically seen in F344/N rats. These differences included a more complex growth pattern with central necrosis even in some adenomas, an increase in tubular growth patterns, increased amounts of eosinophilic basement membrane material or early scirrhous reactions around the neoplasms, and a frequent location of neoplasms in the deep cortex or at the corticomedullary junction. The latter observation suggests a possible origin from the P3 segment of the proximal tubule.

At 2 years, incidences of hyperplasia of the transitional epithelium of the renal pelvis were increased in all exposed groups. Papillomas of the transitional epithelium were present in two males exposed to 938 ppm and one male exposed to 3,750 ppm; none occurred in females. Hyperplasia usually consisted of multiple, exophytic polyploid growths that originated from the transitional epithelium lining the renal papilla and renal pelvis. Papillomas were solid, nodular proliferations of pleomorphic epithelium. Varying amounts of fibrous connective tissue supported the neoplastic growth.

Right kidneys were collected from rats evaluated at 3 months, and α2u-globulin was quantitated in the homogenates using ELISA. Table 8 shows that the concentration of α2u-globulin in the kidney of 3,750 ppm males was greater than that in the control group; in 3,750 ppm females, the concentration of α2u-globulin was less than that in the control group.

TABLE 8
Concentrations of α2u-Globulin in the Supernatant of Kidney Homogenates from Male and Female Rats at the 3-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone

	0 ppm	3,750 ppm
Male		
α2u-Globulin (ng/μg soluble protein)	70 ± 28.9	430 ± 296
Female		
α2u-Globulin (ng/μg soluble protein)	0.555 ± 0.262	0.086 ± 0.074

Urinary Bladder: At 2 years, at least one male in each exposed group had a urinary bladder papilloma; the incidence in the 1,875 ppm group was significantly greater than the control incidence, and the incidences in groups exposed to 938 ppm or greater exceeded the historical control range (Tables 7, A3, and A4c). There was a positive trend in the incidences of papilloma or carcinoma (combined) in females, and the incidence in the 3,750 ppm group exceeded the historical control range (Tables 7, B1, and B4b). Proliferative lesions of the urinary bladder were focal, exophytic growths of the transitional epithelium and occurred in the absence of any other significant lesions. Papillomas were pedunculated lesions with a connective tissue stalk and increased cellular pleomorphism. The single carcinoma was a large nodular lesion with several foci of early squamous differentiation. Several areas of cellular invasion into the underlying connective tissue along with chronic active inflammation were evident along the base of the lesion. The incidences of hyperplasia of the transitional epithelium followed a positive trend in females but were not significantly increased in the exposed groups (Tables 7 and B5). Hyperplasias were small, occasionally nodular lesions composed of increased numbers of epithelial cells without appreciable cellular pleomorphism.

Liver: At the 12-month interim evaluation, liver weights of males and females in the 3,750 ppm groups were significantly greater than those of the control groups (Table G2). Incidences of centrilobular hypertrophy in exposed groups were significantly greater than those in the controls at 3 and 12 months and

at 2 years (except 469 ppm males at 2 years) (Tables 9, A5, and B5). At 2 years, generally significant increases occurred in the incidences of cystic degeneration, inflammation, eosinophilic focus, and mixed cell focus in exposed groups of males and females, cytoplasmic vacuolization in exposed groups of males, and angiectasis in exposed groups of females. Although the incidences were increased in exposed groups, these nonneoplastic lesions were of minimal severity (Plate 5), occupied less than 1% to 5% of the hepatic parenchyma, and were qualitatively similar to spontaneous background lesions seen in the livers of older rats. Incidences of basophilic focus were significantly greater than those in the controls in 469 and 938 ppm males and 469 ppm females, but the incidence in 3,750 ppm females was significantly less. The incidence of hepatocellular adenoma in 938 ppm females at 2 years was significantly greater than the control incidence, and the incidences of hepatocellular adenoma exceeded the historical control range in females exposed to 938 ppm or greater (Tables 9, B3, B4c). In exposed males, incidences of hepatocellular adenoma or carcinoma (combined) were marginally greater than that in the controls, and the incidences were at the upper end of the historical control range (Tables 9, A3, and A4d).

Centrilobular hypertrophy was characterized by increased cellular size and decreased sinusoidal width in centrilobular regions; the nuclei and cytoplasm were larger, and the cytoplasm was finely vacuolated. Cystic degeneration was characterized by one or more cystic areas lacking endothelial lining and containing finely flocculent eosinophilic material. Inflammation tended to be multifocal and consisted primarily of mononuclear inflammatory cells. Foci of cellular alteration were generally round to oval, occasionally irregular in shape, and varied in size from less than one to several lobules in diameter; cellular pleomorphism was evident, but lobular architecture was generally maintained. Cytoplasmic vacuolization did not exhibit a strong lobular preference and consisted of hepatocytes containing several large, clear vacuoles. Angiectasis consisted of irregularly sized, dilated sinusoids containing erythrocytes and lined by a single layer of endothelium. Adenomas were well circumscribed, occupied an area greater than one lobule, and distinctly compressed the adjacent parenchyma; normal lobular architecture was disrupted, central veins and portal tracts were not readily apparent, and cellular atypia and mitotic figures were usually present.

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Male					
3-Month Interim Evaluation					
Number Examined Microscopically	5				5
Centrilobular Hypertrophy[a]	0				5** (2.0)[b]
12-Month Interim Evaluation					
Number Examined Microscopically	5				5
Centrilobular Hypertrophy	0				5** (2.0)
2-Year Study					
Number Examined Microscopically	50	50	50	50	50
Angiectasis	6 (1.0)	21** (1.1)	13 (1.3)	9 (1.1)	9 (1.0)
Basophilic Focus	25	35*	35*	32	23
Eosinophilic Focus	9	22**	30**	29**	20**
Mixed Cell Focus	4	12*	15**	13*	10
Centrilobular Hypertrophy	0	4 (1.0)	21** (1.0)	13** (1.2)	29** (1.1)
Cystic Degeneration	9 (1.0)	31** (1.3)	36** (1.2)	28** (1.3)	29** (1.1)
Inflammation	13 (1.0)	30** (1.0)	28** (1.0)	30** (1.0)	27** (1.0)
Vacuolization Cytoplasmic	5 (2.4)	18** (1.4)	23** (1.2)	17** (1.2)	23** (1.2)
Hepatocellular Adenoma	1	3	4	4	2
Hepatocellular Carcinoma	0	0	0	1	1
Hepatocellular Adenoma or Carcinoma[c]	1	3	4	5	3
Female					
3-Month Interim Evaluation					
Number Examined Microscopically	5				5
Centrilobular Hypertrophy	0				5** (1.8)
12-Month Interim Evaluation					
Number Examined Microscopically	5				5
Centrilobular Hypertrophy	0				5** (2.0)
2-Year Study					
Number Examined Microscopically	50	50	50	50	49
Angiectasis	3 (1.0)	15** (1.2)	18** (1.2)	15** (1.1)	21** (1.1)
Basophilic Focus	37	50**	34	33	15**
Eosinophilic Focus	8	32**	34**	39**	34**
Mixed Cell Focus	3	30**	20**	23**	13*
Cystic Degeneration	0	5* (1.0)	10** (1.2)	10** (1.1)	6* (1.0)
Inflammation	25 (1.0)	46** (1.2)	44** (1.2)	38* (1.1)	46** (1.2)
Centrilobular Hypertrophy	0	18** (1.0)	23** (1.1)	19** (1.1)	26** (1.3)
Hepatocellular Adenoma[d]	0	2	6*	4	3
Hepatocellular Carcinoma	1	0	0	0	0
Hepatocellular Adenoma or Carcinoma	1	2	6	4	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): 26/902 (2.9% ± 3.5%); range, 0%-10%
[d] Historical incidence: 4/901 (0.4% ± 1.1%); range, 0%-4%

Skin: At 2 years, there was a positive trend in the incidences of keratoacanthoma in males (0 ppm, 0/50; 469 ppm, 2/50; 938 ppm, 3/50; 1,875 ppm, 2/50; 3,750 ppm, 5/50; Table A3). Keratoacanthoma occurred somewhat frequently in the skin of male F344/N rats in historical NTP feed studies [40/904 (4.4% ± 3.6%); range, 0%-14%] but was rare in females [1/901 (0.1% ± 0.5%); range, 0%-2%]. Keratoacanthoma is a benign epithelial neoplasm that has been proposed to arise from hair follicles and occurs more often on the back, thorax, or tail. Spontaneous or chemically induced skin neoplasms are generally either of epithelial or mesenchymal origin. Skin neoplasms of epithelial origin are classified in a variety of categories based on histogenesis and histomorphology. There was a chemical-associated increase in the incidence of skin neoplasms of epithelial origin in rats (particularly males) from 10 of the 250 most recent NTP studies (NTP, 1998). In eight of those studies, the increases included a variety of neoplasms of epithelial origin such as basal cell neoplasms, sebaceous gland neoplasms, squamous cell neoplasms, and keratoacanthomas. The positive trend in the incidences of keratoacanthoma in the present study was not considered to be related to anthraquinone exposure because the incidence in the 3,750 ppm group was within the historical control range, and the absence of keratoacanthoma in the control group was uncommon, occurring in only two of 18 other studies from the current historical database. In addition, chemical induction of skin neoplasms usually results in increases in several epithelial neoplasm types; there was no significant increase in the combined incidences of epithelial skin neoplasms in males in this study [(squamous cell papilloma, keratoacanthoma, trichoepithelioma, or basal cell adenoma (combined): 3/50, 3/50, 3/50, 2/50, 7/50; Table A3)].

Thyroid Gland: At 2 years, the incidences of C-cell adenoma or carcinoma (combined) occurred with a positive trend in females (5/50, 4/50, 5/50, 10/50, 10/49; Table B3), and the incidences in the 1,875 and 3,750 ppm groups were at the upper end of the historical control range [109/898 (12.1% ± 4.5%); range, 4%-20%]. Hyperplasia, adenoma, and carcinoma of the C-cells of the thyroid gland are thought to represent a morphologic and biologic continuum, yet there were no increases in the incidences of hyperplasia (21/50, 29/50, 20/50, 18/50, 18/49; Table B5). Because the incidences in the 1,875 and 3,750 ppm groups are within the historical control range, and the incidences of hyperplasia were not increased in exposed groups,

the positive trend in the incidences of thyroid gland C-cell neoplasms was not considered related to anthraquinone exposure.

Spleen: The incidences of congestion at 3 months and at 2 years in 3,750 ppm males and females and pigmentation at 3 months in 3,750 ppm females and at 2 years in exposed males and females were significantly greater than those in the controls (Tables 10, A5, and B5). Congestion characterized as sinusoidal packing or sequestration of erythrocytes within the spleen may have resulted in splenomegaly. At 2 years, the incidences of hematopoietic cell proliferation were significantly increased in males exposed to 469 or 938 ppm and in all exposed groups of females. The incidences of lymphoid follicle atrophy followed a positive trend in males but were not significantly increased in any exposed group.

Bone Marrow: The incidences of hyperplasia were increased in 3,750 ppm males at 12 months and in most groups of exposed rats at 2 years, and the increases were significant in 938 and 1,875 ppm males and in 469 ppm females at 2 years (Tables 10, A5, and B5). The incidences of atrophy were increased in all groups of exposed females at 2 years, but this lesion was not observed in any male groups. Atrophy was focal to multifocal, variable in size, with a well demarcated area of decreased hematopoietic cells and adipocytes. Macrophages were present within the lesion, suggesting an inflammatory component.

TABLE 10
Incidences of Nonneoplastic Lesions of the Spleen and Bone Marrow in Rats in the 2-Year Feed Study of Anthraquinone

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Male					
3-Month Interim Evaluation					
Spleen[a]	5				5
Congestion[b]	0				5** (2.0)[c]
12-Month Interim Evaluation					
Bone Marrow	5				5
Hyperplasia	0				3 (1.0)
2-Year Study					
Spleen	50	50	50	50	50
Congestion	6 (2.0)	35** (1.8)	37** (1.6)	30** (1.8)	31** (1.6)
Pigmentation	12 (1.3)	36** (1.7)	38** (1.4)	33** (1.2)	28** (1.5)
Hematopoietic Cell Proliferation	37 (1.2)	45* (1.4)	44* (1.7)	43 (1.5)	39 (1.4)
Lymphoid Follicle Atrophy	1 (2.0)	0	2 (3.0)	2 (3.0)	6 (2.5)
Bone Marrow	50	50	50	50	50
Hyperplasia	25 (2.4)	28 (2.1)	37* (2.4)	36* (2.2)	33 (2.5)
Female					
3-Month Interim Evaluation					
Spleen	5				5
Congestion	0				5** (2.0)
Pigmentation	0				4* (1.3)
12-Month Interim Evaluation					
Bone Marrow	5				5
Hyperplasia	0				5** (2.8)
2-Year Study					
Spleen	50	50	50	50	49
Congestion	1 (2.0)	46** (1.4)	42** (1.7)	44** (1.9)	45** (2.0)
Pigmentation	33 (1.6)	45** (1.7)	48** (1.8)	48** (1.9)	47** (2.0)
Hematopoietic Cell Proliferation	39 (1.5)	50** (1.9)	47* (1.8)	47* (1.9)	46* (1.9)
Bone Marrow	50	50	50	50	50
Atrophy	4 (1.0)	13* (1.5)	13* (1.4)	11 (1.3)	13* (1.6)
Hyperplasia	19 (2.2)	31* (2.0)	28 (2.0)	19 (2.1)	23 (1.9)

* 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 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

Mononuclear Cell Leukemia: The incidences of mononuclear cell leukemia at 2 years were significantly decreased in all groups of exposed rats and were less than the historical control ranges (Tables 11, A3, A4e, B3, and B4d).

TABLE 11
Incidences of Mononuclear Cell Leukemia in Male and Female Rats in the 2-Year Feed Study of Anthraquinone

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Male					
2-Year Study					
Mononuclear Cell Leukemia[a]					
Overall rate[b]	25/50 (50%)	2/50 (4%)	1/50 (2%)	5/50 (10%)	7/50 (14%)
Adjusted rate[c]	56.4%	4.5%	2.3%	11.4%	16.7%
Terminal rate[d]	12/22 (55%)	0/23 (0%)	0/22 (0%)	3/26 (12%)	3/22 (14%)
First incidence (days)	499	668	705	674	607
Poly-3 test[e]	P=0.003N	P<0.001N	P<0.001N	P<0.001N	P<0.001N
Female					
2-Year Study					
Mononuclear Cell Leukemia[f]					
Overall rate	18/50 (36%)	1/50 (2%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate	38.0%	2.2%	2.2%	4.2%	0.0%
Terminal rate	2/23 (9%)	1/40 (3%)	1/35 (3%)	1/37 (3%)	0/40 (0%)
First incidence (days)	571	730 (T)	730 (T)	634	—[g]
Poly-3 test	P<0.001N	P<0.001N	P<0.001N	P<0.001N	P<0.001N

(T)Terminal sacrifice
[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 is the P value associated with the trend test. Beneath the exposed group incidences 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%
[g] Not applicable; no neoplasms in animal group

MICE

14-WEEK STUDY

All mice survived until the end of the study (Table 12). Final mean body weights, body weight gains, and feed consumption were similar among exposed and control groups. Dietary concentrations of 1,875, 3,750, 7,500, 15,000, or 30,000 ppm anthraquinone resulted in average daily doses of approximately 250, 500, 1,050, 2,150, or 4,300 mg anthraquinone/kg body weight to males and 300, 640, 1,260, 2,600, or 5,300 mg/kg to females. There were no clinical findings related to anthraquinone exposure.

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

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	23.2 ± 0.3	37.5 ± 0.9	14.3 ± 0.8		4.1	4.1
1,875	10/10	23.2 ± 0.2	39.4 ± 0.8	16.2 ± 0.8	105	4.3	4.0
3,750	10/10	23.5 ± 0.3	38.8 ± 0.8	15.3 ± 0.7	104	4.1	4.2
7,500	10/10	23.4 ± 0.3	39.0 ± 0.7	15.6 ± 0.7	104	4.2	4.2
15,000	10/10	23.1 ± 0.3	36.1 ± 0.5	13.0 ± 0.5	96	4.3	4.2
30,000	10/10	23.7 ± 0.2	36.8 ± 0.7	13.1 ± 0.6	98	4.0	4.5
Female							
0	10/10	18.9 ± 0.3	30.0 ± 0.7	11.1 ± 0.6		3.6	3.3
1,875	10/10	19.7 ± 0.2	32.5 ± 0.6	12.8 ± 0.6	108	3.7	3.6
3,750	10/10	19.0 ± 0.3	30.2 ± 0.8	11.3 ± 0.6	101	4.2	3.9
7,500	10/10	19.6 ± 0.4	32.3 ± 0.7	12.7 ± 0.5	108	4.6	3.3
15,000	10/10	19.4 ± 0.2	31.4 ± 0.6	12.0 ± 0.6	105	3.9	3.5
30,000	10/10	19.4 ± 0.2	29.9 ± 0.7	10.5 ± 0.6	100	3.6	3.8

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

Similar to that observed in rats, a responsive anemia occurred in exposed mice at week 14 (Table F2). The anemia was demonstrated by decreased hematocrit values, hemoglobin concentrations, and/or erythrocyte counts in males exposed to 7,500 ppm or greater and in all groups of exposed females. An erythropoietic

response to the anemia was demonstrated by increased reticulocyte counts in 15,000 and 30,000 ppm males and in exposed groups of females. Accompanying increased reticulocyte counts were minimal increases in mean cell volumes and mean cell hemoglobin values. Mean cell hemoglobin concentrations were minimally increased in 15,000 and 30,000 ppm males and females. There were minimal to mild exposure concentration-related increases in platelet counts in 15,000 and 30,000 ppm males and all exposed groups of females.

Liver weights were significantly greater than those of the control groups in all exposed groups of male and female mice, as were the kidney weights of 30,000 ppm males (Table G3).

No differences in epididymal spermatozoal measurements or estrous cycle lengths were observed between exposed and control groups (Tables H3 and H4).

Several treatment-related histologic lesions were observed in male and female mice (Table 13). The incidences of centrilobular hypertrophy in the liver of males and females exposed to 3,750 ppm or greater were significantly greater than those in the controls, and the severities increased with increasing exposure concentration. Affected centrilobular hepatocytes exhibited slightly enlarged nuclei and an increased amount of cytoplasm that was more eosinophilic and less granular than that seen in normal hepatocytes. Significantly increased incidences of cytoplasmic alteration, which was characterized by the presence of bright eosinophilic granules, occurred in the transitional epithelial cells of the urinary bladder in all exposed males and females, and the severities increased with increasing exposure concentration. The incidences of hematopoietic cell proliferation were increased in all groups of exposed males and females. Minimal to mild pigmentation was observed in the spleen of all exposed mice except one 30,000 ppm male and one 30,000 ppm female. Neither lesion was observed in control males. All control females and 1,875 ppm males exhibited minimal pigmentation; the severities of pigmentation were mild in all remaining exposed groups of mice.

TABLE 13
Incidences of Selected Nonneoplastic Lesions in Mice in the 14-Week Feed Study of Anthraquinone

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
Male						
Liver[a]	10	10	10	10	10	10
Centrilobular Hypertrophy[b]	0	1 (1.0)[c]	9** (1.6)	10** (2.8)	10** (3.0)	10** (3.1)
Urinary Bladder	10	10	10	10	10	10
Transitional Epithelium, Cytoplasmic Alteration	0	10** (1.1)	10** (2.5)	10** (3.1)	10** (3.2)	10** (3.8)
Spleen	10	10	10	10	10	10
Hematopoietic Cell Proliferation	0	6** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	9** (1.0)
Pigmentation	0	10** (1.2)	10** (1.8)	10** (2.0)	10** (2.0)	9** (2.0)
Female						
Liver	10	10	10	10	10	10
Centrilobular Hypertrophy	0	2 (1.0)	5* (1.0)	9** (1.1)	7** (1.7)	10** (2.4)
Urinary Bladder	10	10	10	10	10	10
Transitional Epithelium, Cytoplasmic Alteration	0	10** (1.0)	10** (1.0)	10** (1.7)	10** (2.8)	10** (3.5)
Spleen	10	10	10	10	9	10
Hematopoietic Cell Proliferation	6 (1.0)	9 (1.8)	10* (1.7)	10* (1.8)	9 (2.0)	9 (2.0)
Pigmentation	10 (1.0)	10 (2.0)	10 (2.0)	10 (2.0)	9 (2.0)	9 (2.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ 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

Exposure Concentration Selection Rationale: The primary exposure concentration-limiting response was observed in the liver. Liver weights were significantly increased in all exposed groups of male and female mice. Incidences of centrilobular hypertrophy were significantly increased in males and females exposed to 3,750 ppm or greater; severities increased with increasing exposure concentration in females but remained relatively constant in males exposed to 7,500 ppm or greater. Based on this response, particularly in males, 7,500 ppm was considered an adequate high exposure concentration for a 2-year study. Lower exposure concentrations of 833 and 2,500 ppm were selected to provide a wide exposure range.

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 4). Survival of 7,500 ppm male mice was significantly less than the survival of the control group; survival of other exposed groups of males and females was similar to the controls.

TABLE 14
Survival of Mice in the 2-Year Feed Study of Anthraquinone

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	3	3	3	8
Natural deaths	2	6	4	19
Animals surviving to study termination	45	41	43	23
Percent probability of survival at end of study[a]	90	82	86	46
Mean survival (days)[b]	709	709	711	668
Survival analysis[c]	P<0.001	P=0.374	P=0.751	P<0.001
Female				
Animals initially in study	50	50	50	50
Accidental deaths[d]	0	0	3	0
Missing[d]	0	0	0	1
Moribund	6	3	4	2
Natural deaths	9	5	8	5
Animals surviving to study termination	35	42	35	42
Percent probability of survival at end of study	70	84	76	86
Mean survival (days)	695	709	699	695
Survival analysis	P=0.227N	P=0.175N	P=0.737N	P=0.117N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] 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 vehicle controls are in the exposed columns. A negative trend or lower mortality in an exposed group is indicated by **N**.
[d] Censored from survival analyses

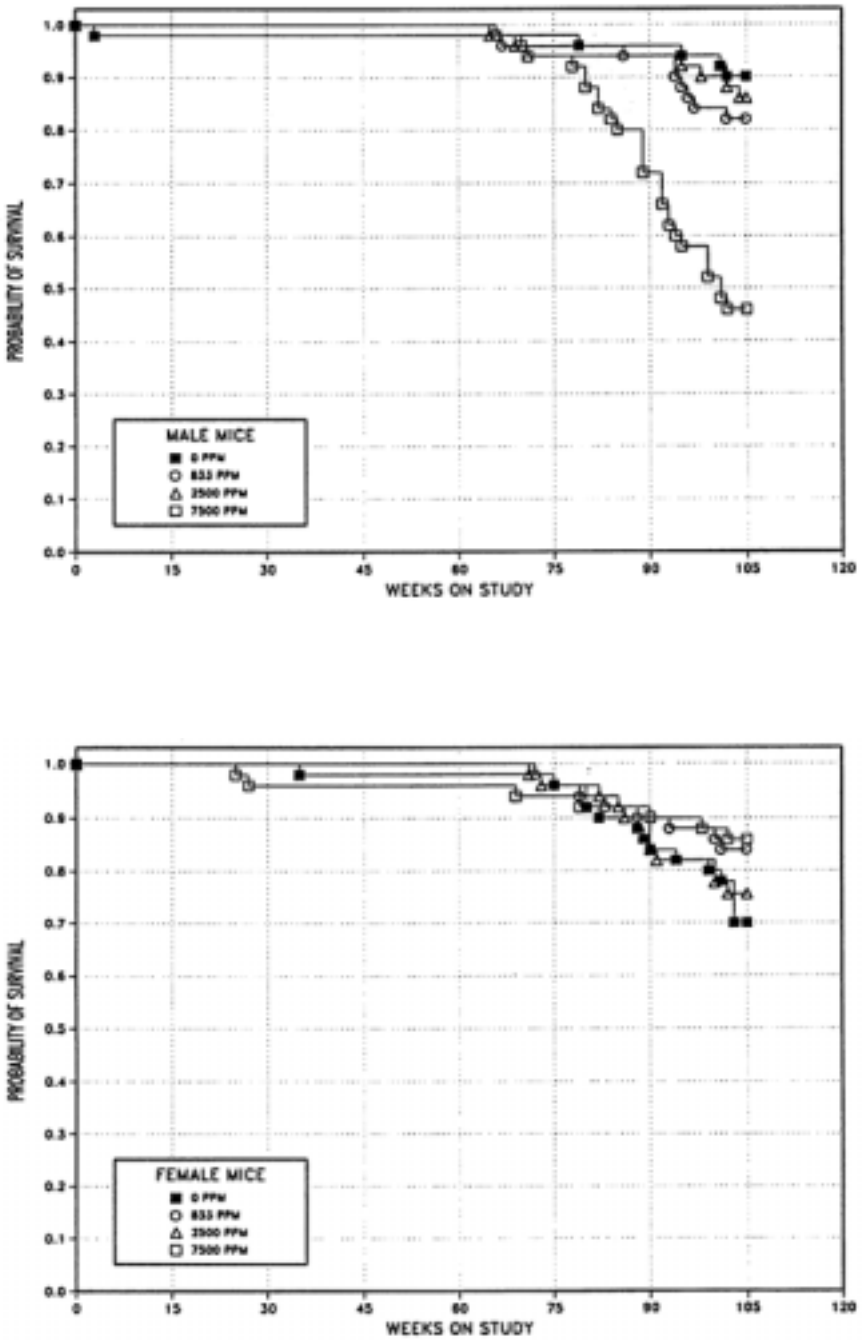


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Anthraquinone in Feed for 2 Years

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of 7,500 ppm males from week 86 and of 7,500 ppm females from week 98 were less than those of the control groups (Tables 15 and 16 and Figure 5). Feed consumption by exposed groups of males and females was similar to that by the controls (Tables K3 and K4). Dietary concentrations of 833, 2,500, or 7,500 ppm anthraquinone delivered average daily doses of approximately 90, 265, or 825 mg anthraquinone/kg body weight to males and 80, 235, or 745 mg/kg to females. There were no clinical findings that could be related to chemical exposure.

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

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	833 ppm Av. Wt. (g)	833 ppm Wt. (% of controls)	833 ppm No. of Survivors	2,500 ppm Av. Wt. (g)	2,500 ppm Wt. (% of controls)	2,500 ppm No. of Survivors	7,500 ppm Av. Wt. (g)	7,500 ppm Wt. (% of controls)	7,500 ppm No. of Survivors
1	21.7	50	21.8	101	50	21.8	101	50	21.7	100	50
2	23.0	50	23.2	101	50	23.2	101	50	23.4	102	50
6	28.1	49	28.7	102	50	28.6	102	50	28.4	101	50
10	32.1	49	33.3	104	50	33.4	104	50	32.6	102	50
14	35.5	49	37.0	104	50	36.6	103	50	35.8	101	50
18	38.7	49	40.5	105	50	40.1	104	50	39.4	102	50
22	41.6	49	43.3	104	50	42.6	102	50	41.8	101	50
26	43.3	49	44.6	103	50	43.8	101	50	42.9	99	50
30	45.4	49	46.1	102	50	45.9	101	50	45.0	99	50
34	46.4	49	47.1	102	50	47.2	102	50	46.1	99	50
38	47.3	49	47.6	101	50	47.4	100	50	46.8	99	50
42	48.2	49	48.5	101	50	48.1	100	50	47.9	99	50
46	48.1	49	48.3	100	50	48.2	100	50	47.9	100	50
50	49.4	49	50.3	102	50	50.3	102	50	49.6	100	50
54	48.4	49	49.5	102	50	50.0	103	50	49.0	101	50
58	49.1	49	49.9	102	50	50.5	103	50	49.8	101	50
61	50.1	49	50.6	101	50	50.1	100	50	50.5	101	50
66	49.4	49	49.9	101	50	50.3	102	49	50.8	103	49
70	49.6	49	50.2	101	48	50.5	102	48	49.7	100	49
74	49.4	49	50.9	103	48	50.3	102	47	50.4	102	47
78	49.3	49	50.7	103	48	50.2	102	47	48.6	99	46
82	49.1	48	50.2	102	48	49.6	101	47	46.8	95	44
86	49.0	48	50.0	102	48	49.7	101	47	46.2	94	40
90	48.5	48	49.8	103	47	49.0	101	47	44.0	91	36
94	48.6	48	48.2	99	47	48.3	99	47	41.7	86	31
98	48.4	47	48.1	99	42	47.6	98	46	40.0	83	29
102	47.8	46	46.3	97	42	46.0	96	45	37.9	79	24
Mean for weeks											
1-13	26.2		26.8	102		26.8	102		26.5	101	
14-52	44.4		45.3	102		45.0	102		44.3	100	
53-102	49.0		49.6	101		49.4	101		46.6	95	

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

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	833 ppm Av. Wt. (g)	833 ppm Wt. (% of controls)	833 ppm No. of Survivors	2,500 ppm Av. Wt. (g)	2,500 ppm Wt. (% of controls)	2,500 ppm No. of Survivors	7,500 ppm Av. Wt. (g)	7,500 ppm Wt. (% of controls)	7,500 ppm No. of Survivors
1	17.0	50	17.0	100	50	17.0	100	50	17.4	102	50
2	18.4	50	17.5	95	50	18.6	101	50	18.9	103	50
6	22.9	50	22.3	97	50	23.1	101	50	23.4	102	50
10	26.5	50	26.5	100	50	26.3	99	50	27.7	105	50
14	29.5	50	29.4	100	50	30.2	102	50	30.6	104	50
18	33.7	50	33.6	100	50	34.3	102	50	34.6	103	50
22	36.4	50	36.6	101	50	36.5	100	50	36.7	101	50
26	39.0	50	38.4	99	50	38.4	99	50	37.7	97	49
30	41.4	50	40.8	99	50	41.3	100	50	39.7	96	48
34	43.8	50	43.6	100	50	43.4	99	50	42.8	98	48
38	45.4	49	45.1	99	50	45.0	99	50	44.0	97	48
42	47.2	49	47.0	100	50	46.6	99	50	45.4	96	48
46	48.5	49	48.2	99	50	47.7	98	50	47.1	97	48
50	50.8	49	50.2	99	50	49.7	98	50	48.4	95	48
54	51.3	49	51.4	100	50	50.8	99	50	49.7	97	48
58	52.1	49	50.7	97	50	51.1	98	50	49.6	95	48
61	53.3	49	53.1	100	50	53.0	99	50	50.8	95	48
66	53.5	49	54.0	101	50	54.3	102	50	50.6	95	48
70	54.5	49	54.2	99	50	55.1	101	50	52.9	97	47
74	55.9	49	55.3	99	49	56.2	101	48	54.4	97	47
78	56.1	48	56.0	100	48	56.5	101	48	53.6	96	47
82	56.0	46	56.0	100	47	56.9	102	47	54.6	98	46
86	56.1	45	55.8	100	46	55.3	99	46	55.3	99	46
90	56.5	43	56.8	101	45	55.8	99	44	54.5	97	46
94	55.8	42	56.6	101	44	56.5	101	41	54.1	97	45
98	56.8	41	56.3	99	44	54.2	95	38	53.2	94	44
102	54.8	39	55.2	101	42	53.2	97	36	51.3	94	43
Mean for weeks											
1-13	21.2		20.8	98		21.3	100		21.9	103	
14-52	41.6		41.3	100		41.3	100		40.7	98	
53-102	54.8		54.7	100		54.5	100		52.7	96	

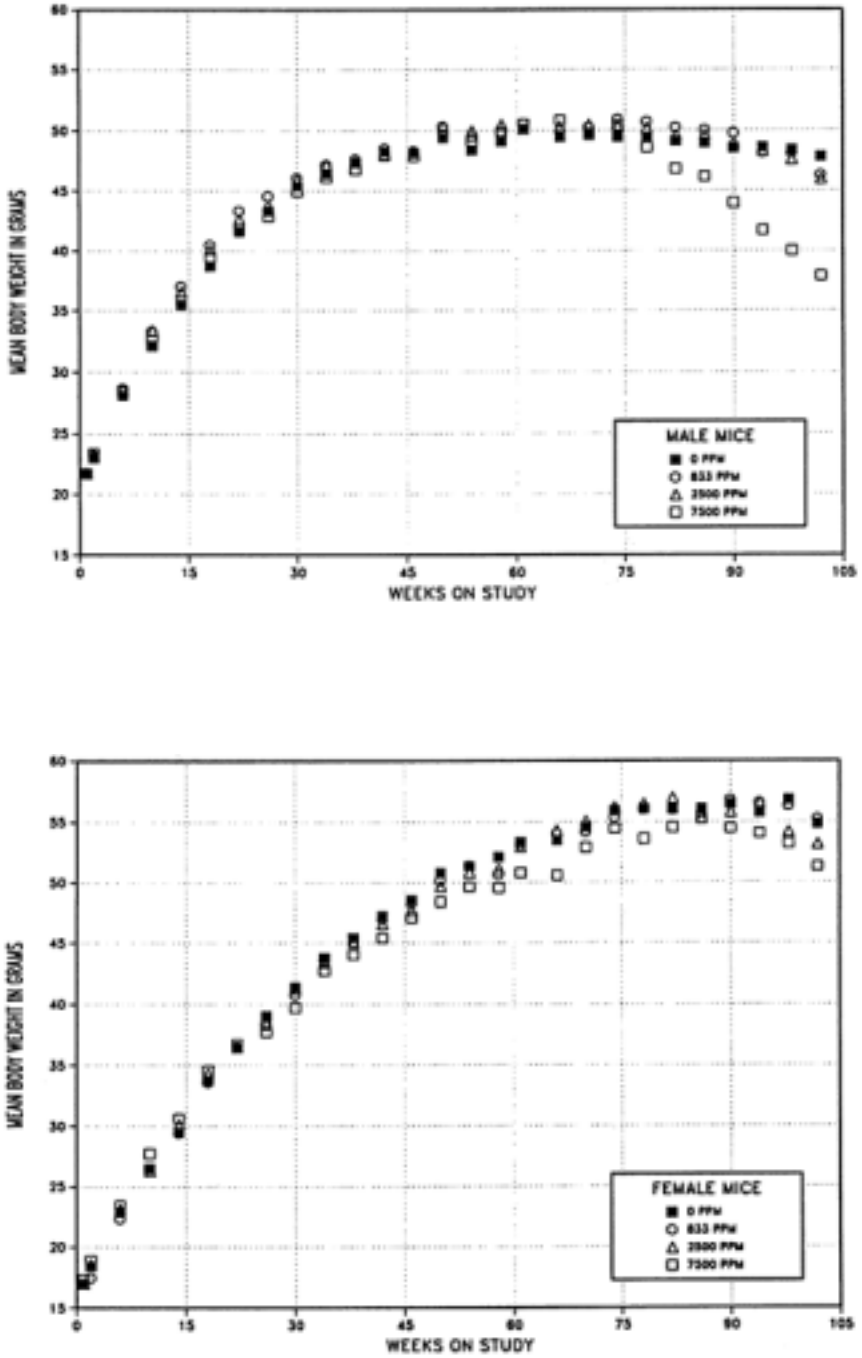


FIGURE 5
Growth Curves for Male and Female Mice Exposed to Anthraquinone in Feed for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, thyroid gland, urinary bladder, spleen, kidney, and pancreatic islets. 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.

Liver: Incidences of hepatocellular neoplasms increased with a positive trend in male and female mice, and the incidences were increased in all exposed groups (Tables 17, C3, and D3). The incidences of multiple hepatocellular adenoma were increased in all exposed groups of mice. Incidences of hepatoblastoma were significantly increased in males exposed to 2,500 or 7,500 ppm (incidences of multiple hepatoblastoma also increased), and one occurred in a 7,500 ppm female. The incidences of liver neoplasms in exposed groups generally exceeded the historical control ranges (Tables 17, C4a, and D4a). Hepatocellular adenomas were well circumscribed lesions occupying an area greater than one hepatic lobule and causing distinct compression of the surrounding parenchyma. Hepatocellular carcinomas were larger than adenomas, not always well demarcated, and exhibited abnormal growth patterns and cellular atypia. Hepatoblastomas were well demarcated, expansive neoplasms frequently exhibiting local invasion and often found within or adjacent to hepatocellular adenomas or carcinomas. Neoplasms were often irregular with blood-filled cystic spaces. Hepatoblasts are small cells with irregular, hyperchromatic nuclei and scant basophilic cytoplasm and diagnostic of hepatoblastomas.

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Male				
Number Examined Microscopically	50	50	50	49
Centrilobular, Hypertrophy[a]	24 (1.3)[b]	34* (1.5)	41** (1.8)	33** (2.5)
Degeneration, Fatty, Focal	0	7** (1.0)	6* (1.5)	0
Eosinophilic Focus	14	17	24*	20*
Hepatocyte, Erythrophagocytosis	1 (1.0)	9** (1.2)	13** (1.4)	6* (1.7)
Hematopoietic Cell Proliferation	0	2 (2.5)	0	4* (1.0)
Necrosis, Focal	2 (1.5)	3 (1.0)	3 (1.7)	8* (2.1)
Hepatocellular Adenoma, Multiple	5	22**	28**	31**
Hepatocellular Adenoma (includes multiple)	21	32*	38**	41**
Hepatocellular Carcinoma, Multiple	1	4	5	9*
Hepatocellular Carcinoma (includes multiple)	8	13	17*	21**
Hepatocellular Adenoma or Carcinoma (includes multiple)[c]				
Overall rate[d]	25/50 (50%)	34/50 (68%)	41/50 (82%)	46/49 (94%)
Adjusted rate[e]	51.7%	70.5%	86.0%	96.1%
Terminal rate[f]	23/45 (51%)	30/41 (73%)	39/43 (91%)	23/23 (100%)
First incidence (days)	662	464	662	456
Poly-3 test[g]	P<0.001	P=0.043	P<0.001	P<0.001
Hepatoblastoma, Multiple	0	1	0	16**
Hepatoblastoma (includes multiple)[h]	1	6	11**	37**
Hepatocellular Carcinoma or Hepatoblastoma (includes multiple)				
Overall rate	9/50 (18%)	18/50 (36%)	27/50 (54%)	45/49 (92%)
Adjusted rate	18.7%	37.3%	56.2%	92.7%
Terminal rate	8/45 (18%)	15/41 (37%)	26/43 (61%)	20/23 (87%)
First incidence (days)	702	464	481	456
Poly-3 test	P<0.001	P=0.033	P<0.001	P<0.001
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma (includes multiple)[c]				
Overall rate	26/50 (52%)	35/50 (70%)	43/50 (86%)	48/49 (98%)
Adjusted rate	53.8%	72.2%	88.9%	98.9%
Terminal rate	24/45 (53%)	30/41 (73%)	40/43 (93%)	23/23 (100%)
First incidence (days)	662	464	481	456
Poly-3 test	P<0.001	P=0.045	P<0.001	P<0.001

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Female				
Number Examined Microscopically	49	50	50	49
Centrilobular, Hypertrophy	1 (1.0)	27** (1.2)	22** (1.2)	39** (1.5)
Degeneration, Fatty, Focal	2 (1.5)	3 (1.7)	1 (2.0)	9* (1.2)
Eosinophilic Focus	6	15*	11	22**
Hepatocellular Adenoma, Multiple	1	17**	13**	30**
Hepatocellular Adenoma (includes multiple)	6	28**	27**	40**
Hepatocellular Carcinoma, Multiple	0	2	1	2
Hepatocellular Carcinoma (includes multiple)	2	3	8	8*
Hepatocellular Adenoma or Carcinoma (includes multiple)[i]				
Overall rate	6/49 (12%)	30/50 (60%)	30/50 (60%)	41/49 (84%)
Adjusted rate	13.4%	63.8%	64.2%	89.3%
Terminal rate	4/35 (11%)	29/42 (69%)	24/35 (69%)	38/42 (91%)
First incidence (days)	519	611	568	549
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatoblastoma	0	0	0	1
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma (includes multiple)[i]				
Overall rate	6/49 (12%)	30/50 (60%)	30/50 (60%)	41/49 (84%)
Adjusted rate	13.4%	63.8%	64.2%	89.3%
Terminal rate	4/35 (11%)	29/42 (69%)	24/35 (69%)	38/42 (91%)
First incidence (days)	519	611	568	549
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

* Significantly different (P≤0.05) from the control group by the Poly-3 test
** P≤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): 440/850 (51.8% ± 8.3%); range, 40%-68%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidences are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence: 0/850
[i] Historical incidence: 273/852 (32.0% ± 9.6%); range, 18%-56%

The incidences of several nonneoplastic lesions of the liver were increased in exposed groups of mice; these lesions were more numerous in males than in females (Tables 17, C5, and D5). Hepatocellular hypertrophy characterized by an increased volume of finely vacuolated cytoplasm, increased nuclear size, and decreased sinusoidal width was increased in frequency and severity in exposed groups of males and females. Focal fatty degeneration, characterized by large clear vacuoles within the hepatocyte cytoplasm, was greater in 7,500 ppm females than in the controls. Several exposed males, but not females, had an unusual change

consisting of clusters of two or more hepatocytes that had cytoplasm markedly distended with erythrocytes (Plate 3). Diagnosed as hepatocyte erythrophagocytosis, this lesion frequently phagocytosized erythrocytes, eccentrically displaced hepatocyte nuclei and caused margination of hepatocellular cytoplasm. Foci of cellular alteration were localized lesions, round to oval but occasionally irregular in shape, that varied in size from less than one up to several hepatic lobules in diameter. Some cellular pleomorphism may have been evident, but lobular architecture was maintained. Hepatocytes in foci usually resembled those found in adjacent normal liver, although cellular atypia and mitotic figures may have been present.

As is frequently observed in NTP studies in which robust liver neoplasm responses occur in mice, the incidences of hepatoblastoma were increased in males. As in the present study, increases are generally most pronounced in male mice. Hepatoblastomas are uncommon neoplasms that occur spontaneously or may be chemically induced in the liver of several strains (Turusov *et al.*, 1973; Nonoyama *et al.*, 1988) of mice. Hepatoblastomas are malignant, and in NTP studies, their metastatic potential appears similar to that of hepatocellular carcinomas. Hepatoblastomas almost always occur within an existing proliferative lesion, most often within a hepatocellular carcinoma, and when that occurs in NTP studies, the entire proliferative lesion is diagnosed as a hepatoblastoma. Although the cell of origin is not definitely known and the biology of these neoplasms is not fully understood, the hepatoblastoma is considered to be part of the spectrum of neoplasms that occurs spontaneously and as a result of chemical treatment. Therefore, while statistical analyses of individual neoplasms are informative, NTP considers analyses of hepatocellular carcinoma or hepatoblastoma (combined) and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) to be the most important in evaluating the hepatocarcinogenic potential of an agent.

Thyroid Gland: Follicular cell adenomas were present in two males each in the 2,500 and 7,500 ppm groups; however, these incidences were not significantly greater than the control incidence and were within the historical control range (Tables 18, C1, and C4b). Follicular cell adenomas were present in all groups of females; however, two 7,500 ppm females had follicular cell carcinomas (Tables 18 and D1). The incidences of follicular cell carcinoma and follicular cell adenoma or carcinoma (combined) in 7,500 ppm females exceeded the historical control ranges (Tables 18 and D4b). The incidences of follicular cell hyperplasia in 2,500 and 7,500 ppm males were significantly greater than those in the controls; the incidences of follicular cell hyperplasia were increased in exposed groups of females, but the differences from controls were not statistically significant.

Follicular cell hyperplasia was characterized as a focal to multifocal change consisting of enlarged follicles lined by increased numbers of follicular epithelial cells. Because of increased cellularity, some papillary infoldings were present in more severe cases. The epithelial cells tended to be slightly hypertrophied but otherwise normal in appearance.

Urinary Bladder: The incidences of intracytoplasmic inclusion body of the urinary bladder were significantly increased in all exposed groups of males (0 ppm, 0/50; 833 ppm, 46/49; 2,500 ppm, 46/49; 7,500 ppm, 42/45; Table C5) and females (0/44, 40/48, 43/46, 46/48; Table D5). Inclusion bodies were eosinophilic granules within the transitional epithelial cells lining the urinary bladder (Plate 4). The granules tended to be multiple, small, and varied in size, and they were PAS positive; only luminal cells were affected.

TABLE 18
Incidences of Nonneoplastic Lesions of the Thyroid Gland in Mice in the 2-Year Feed Study of Anthraquinone

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Male				
Number Examined Microscopically	50	50	49	46
Follicular Cell Hyperplasia[a]	7 (1.3)[b]	10 (1.4)	15* (1.1)	21** (1.2)
Follicular Cell Adenoma[c]	0	0	2	2
Female				
Number Examined Microscopically	45	48	48	48
Follicular Cell Hyperplasia	10 (1.7)	14 (1.2)	16 (1.2)	15 (1.5)
Follicular Cell Adenoma	1	1	2	2
Follicular Cell Carcinoma[d]	0	0	0	2
Follicular Cell Adenoma or Carcinoma[e]				
Overall rate[f]	1/45 (2%)	1/48 (2%)	2/48 (4%)	4/48 (8%)
Adjusted rate[g]	2.4%	2.2%	4.6%	9.1%
Terminal rate[h]	1/35 (3%)	1/42 (2%)	2/35 (6%)	4/42 (10%)
First incidence (days)	730 (T)	730 (T)	730 (T)	730 (T)
Poly-3 test[i]	P=0.078	P=0.741N	P=0.519	P=0.198

* Significantly different (P≤0.05) from the control group by the Poly-3 test
** P≤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): 12/846 (1.4% ± 1.6%); range, 0%-4%
[d] Historical incidence: 2/847 (0.2% ± 0.7%); range, 0%-2%
[e] Historical incidence: 15/847 (1.8% ± 1.7%); range, 0%-6%
[f] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically
[g] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[h] Observed incidence at terminal kill
[i] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidences are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposure group is indicated by **N**.

Other Organs: Incidences of hematopoietic cell proliferation of the spleen in males (12/50, 14/50, 12/49, 30/42; Table C5) and females (9/45, 17/49, 17/48, 26/48; Table D5) exposed to 7,500 ppm were significantly greater than those in the control groups. Pigmentation in the kidney was significantly greater in 7,500 ppm males (0/50, 2/49, 2/49, 18/47; Table C5). The incidence of pancreatic islet hyperplasia in 7,500 ppm females was significantly greater (6/50, 13/50, 10/50, 14/49; Table D5); however, the incidences in 2,500 and 7,500 ppm males were significantly less (40/50, 40/50, 29/50, 17/42; Table C5).

GENETIC TOXICOLOGY

Anthraquinone (97% pure) (33 to 2,500 μg/plate) was mutagenic in *Salmonella typhimurium* strains TA98 and TA100, with and without hamster or rat liver S9 enzymes (Zeiger *et al.,* 1988; Table E1). Subsequent testing with a 100% pure sample of anthraquinone (100 to 10,000 μg/plate) showed no detectable mutagenic response in TA98, TA100, or TA102, with or without 10% rat liver S9 (Table E2). Additional testing of several substituted anthraquinones revealed an interesting pattern of responses. 1-Hydroxyanthraquinone (up to 10,000 μg/plate) was not mutagenic in TA98, TA100, or TA102, with or without rat liver S9 (Table E3). 2-Hydroxyanthraquinone (3.3 to 450 μg/plate) was mutagenic at low doses in TA98 in the absence of rat liver S9; it was not reproducibly mutagenic with S9, and no mutagenic response was seen with this compound in TA100, with or without S9 (Table E4). 1-, 2-, and 9-Nitroanthracene were all mutagenic in TA98 and TA100, with and without 10% rat liver S9 (Tables E5, E6, and E7); based on the magnitudes of the responses and the lowest effective concentrations required to produce a clear increase in mutant colonies, 2-nitroanthracene was the strongest mutagen of these three substituted anthracenes. 9-nitroanthracene was more strongly mutagenic with S9 than without S9; both trials in TA100 conducted in the absence of S9 were positive, but the peak response was less than twice the control frequency. In contrast to the pattern of mutagenicity seen with 9-nitroanthracene, 1-nitroanthracene produced responses of similar magnitude with and without S9 while 2-nitroanthracene was clearly more mutagenic without S9.

Negative results were obtained in an acute bone marrow micronucleus test performed with male mice administered 500 to 2,000 mg/kg anthraquinone via intraperitoneal injection (Table E8). However, when male and female mice administered anthraquinone (99.9% pure) in feed (1,875 to 30,000 ppm) for 14 weeks were examined for frequency of micronucleated normochromatic erythrocytes in the peripheral blood, significant increases over the control frequencies were noted in male and female mice at the highest exposure concentration (Table E9). Although only the 30,000 ppm female group differed significantly from the control frequency by pairwise comparison, both data sets yielded positive trend tests, and the peripheral

blood micronucleus test was judged to be positive for both male and female mice. **Evidence of increased erythropoiesis in treated mice was demonstrated by the slightly elevated percent PCE values in several of the exposure groups, mostly in exposed female mice. The data do not demonstrate a direct correlation between percent PCEs and micronucleus frequency except in the high exposure concentration groups where both male and female mice showed the highest frequencies of micronucleated erythrocytes and the highest percent PCE values. An increased rate of erythropoiesis may have contributed to the micronucleus responses seen in the high exposure concentration groups, because increased cell proliferation can produce increased levels of mitotic errors.**

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

A physiologically based pharmacokinetic (PBPK) model resulting from oral exposure was developed to characterize tissue concentrations of anthraquinone in rats (Appendix I). The PBPK model consists of a series of mass balance differential equations that represent, in quantitative terms, the physiological and biochemical processes that affect the behavior of anthraquinone in exposed rats. As shown in Figure 6, the rat is represented as separate tissue compartments including the sites of oral absorption and the sites where anthraquinone is subsequently stored or metabolized. By solving the equations in this model simultaneously, estimates of tissue concentration time courses of anthraquinone are generated for any simulated exposure. Therefore, the PBPK model can be used to relate tissue dosimetry to adverse effects resulting from exposure to anthraquinone.

Data used to create the anthraquinone PBPK model were obtained from the literature or from the current rat study. Rat-specific physiologic parameters including cardiac output, blood and organ volumes, organ blood perfusion rates, and stomach and intestinal transit times were obtained from the literature. Tissue/plasma partition coefficients for anthraquinone were estimated from a regression equation of tissue solubility in relation to tissue lipid concentration and K_{ow} for aromatic compounds. Plasma time-course data were generated in conjunction with the current 14-week and 2-year studies. Data on plasma concentrations of

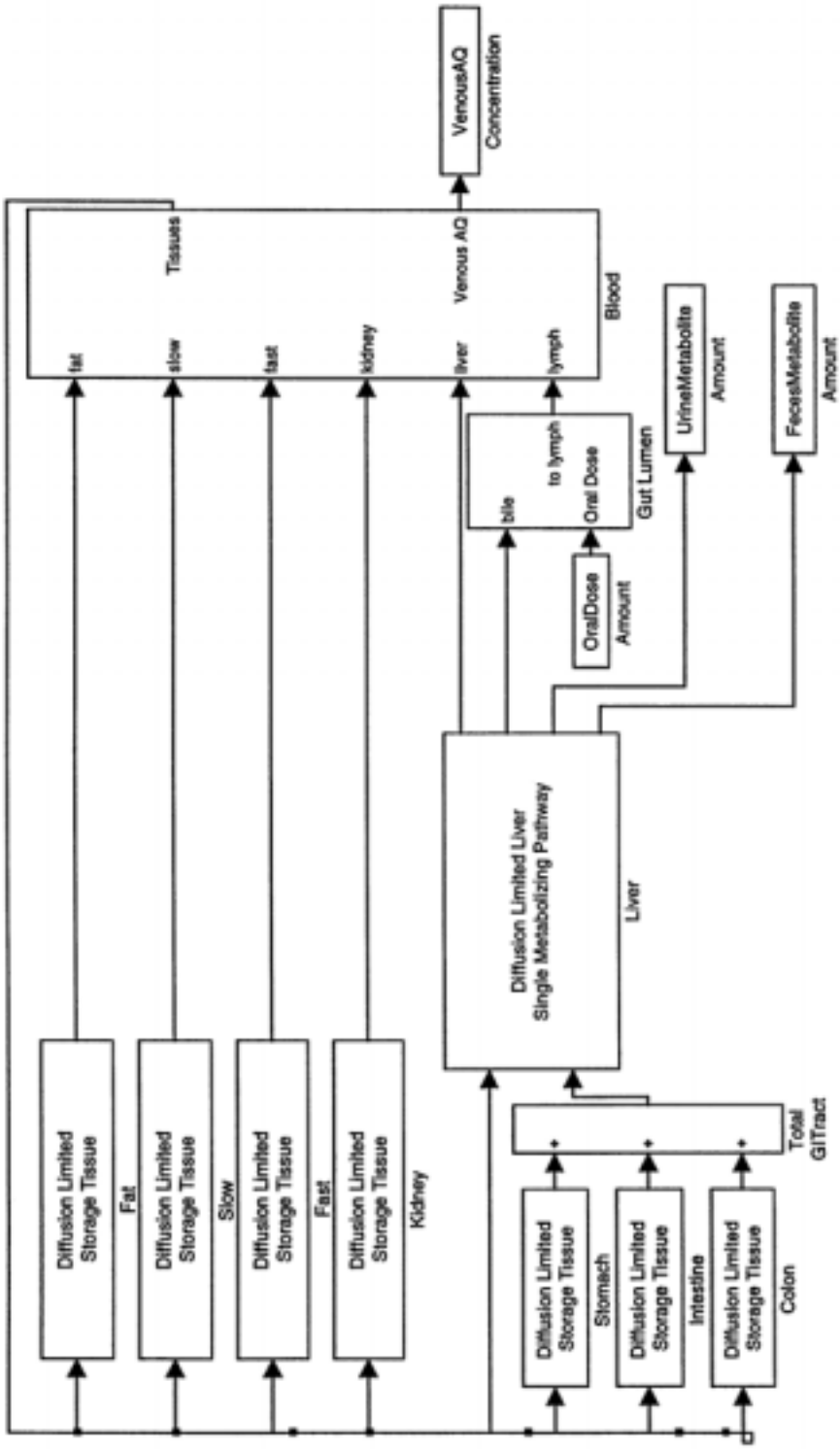


FIGURE 6
Schematic Representation of the Physiologically Based Pharmacokinetic Model for Anthraquinone

anthraquinone in rats administered anthraquinone in feed for 8 days and 3, 6, 12, and 18 months are presented in Appendix I; data on rats administered anthraquinone by a single intravenous injection or by a single oral gavage dose are presented in Appendix N. These data were used to model the absorption, distribution to tissues and organs, and metabolic elimination of anthraquinone by rats exposed to dosed feed.

Fitting the PBPK model to the intravenous data provides an initial characterization of the kinetics of distribution and metabolic elimination of anthraquinone in rats, while the single-dose gavage data permit description of the kinetics of absorption of anthraquinone after oral administration as well as its distribution and metabolic elimination. Metabolism modeling was limited to the liver because there are no data available to characterize extrahepatic metabolism of anthraquinone. The optimized fits of the PBPK model to the intravenous and gavage plasma time-course data for rats are shown in Figures 7 and 8. The log-weighted sum of squared errors for the fit of the model simulations to the experimental data was 10.9 for males and 14.0 for females. The PBPK model included eight adjustable parameters: K_{abs} and V_{abs} to quantify the rates of saturable intestinal absorption of anthraquinone; V_{max} and K_m to quantify rates of hepatic metabolism; $Perm_{discon}$ and $Perm_{cont}$ to quantify permeabilities of organs with discontinuous or continuous capillary barriers; k_{bile} to quantify first-order biliary elimination; and PC_{mult} to quantify the partition coefficients of the tissues. The optimized values for these parameters are shown in Table 19.

The optimal fit of the anthraquinone model to the plasma time-course data indicates that oral absorption of anthraquinone is delayed and incomplete. Absorption was assumed to occur slowly from the small intestine of rats by a saturable process because variant models that had different absorption attributes gave a worse fit to the experimental data. Packaged in chylomicrons, anthraquinone is likely taken up by the lymph that drains the small intestine and than passed into the mixed venous blood; consequently, it does not undergo first-pass liver metabolism. The data also indicate that anthraquinone is distributed slowly to tissues by a diffusion-limited transport process, is stored in fatty tissues due to its high lipophilicity, and is slowly metabolized by a saturable kinetic process.

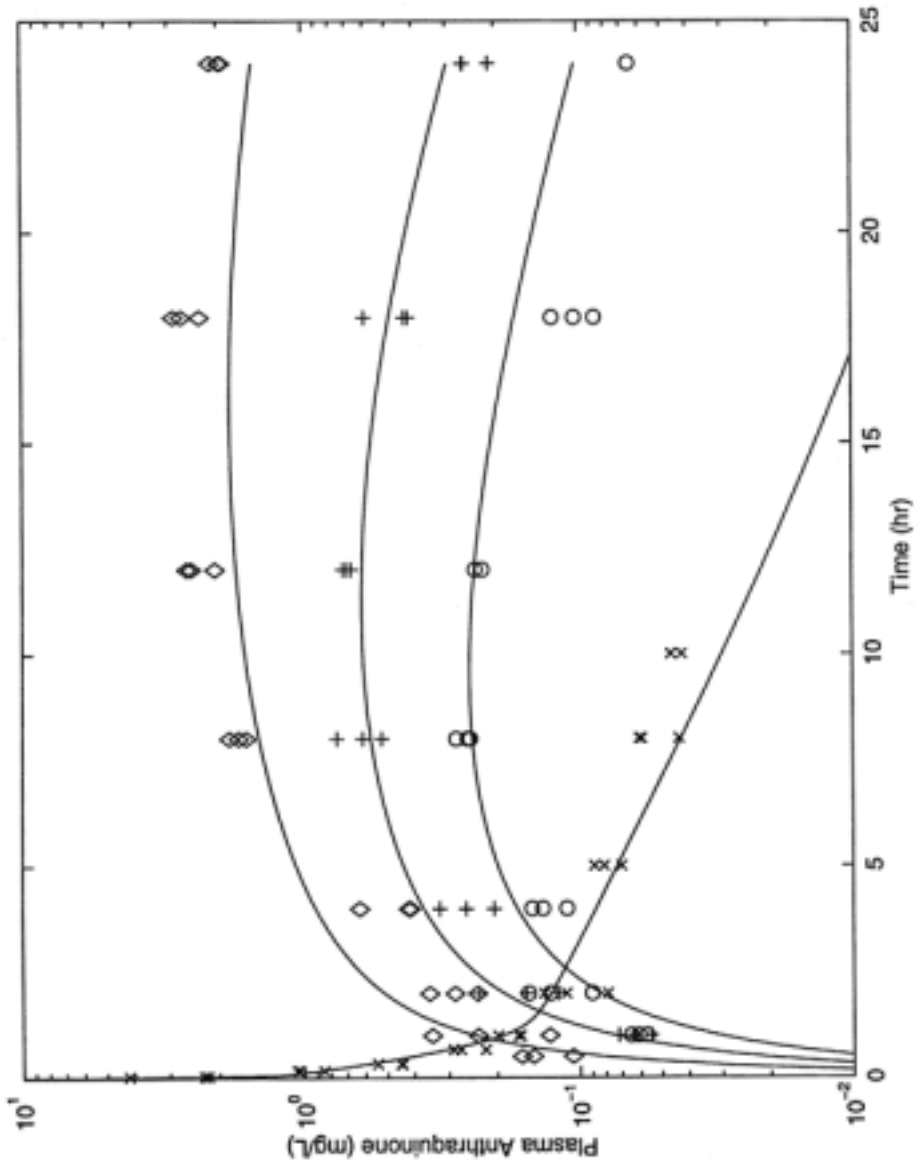


FIGURE 7
Plasma Anthraquinone Concentrations in Male Rats after a Single Intravenous or Gavage Dose. The solid lines represent the fit of these data to the physiologically based pharmacokinetic model.

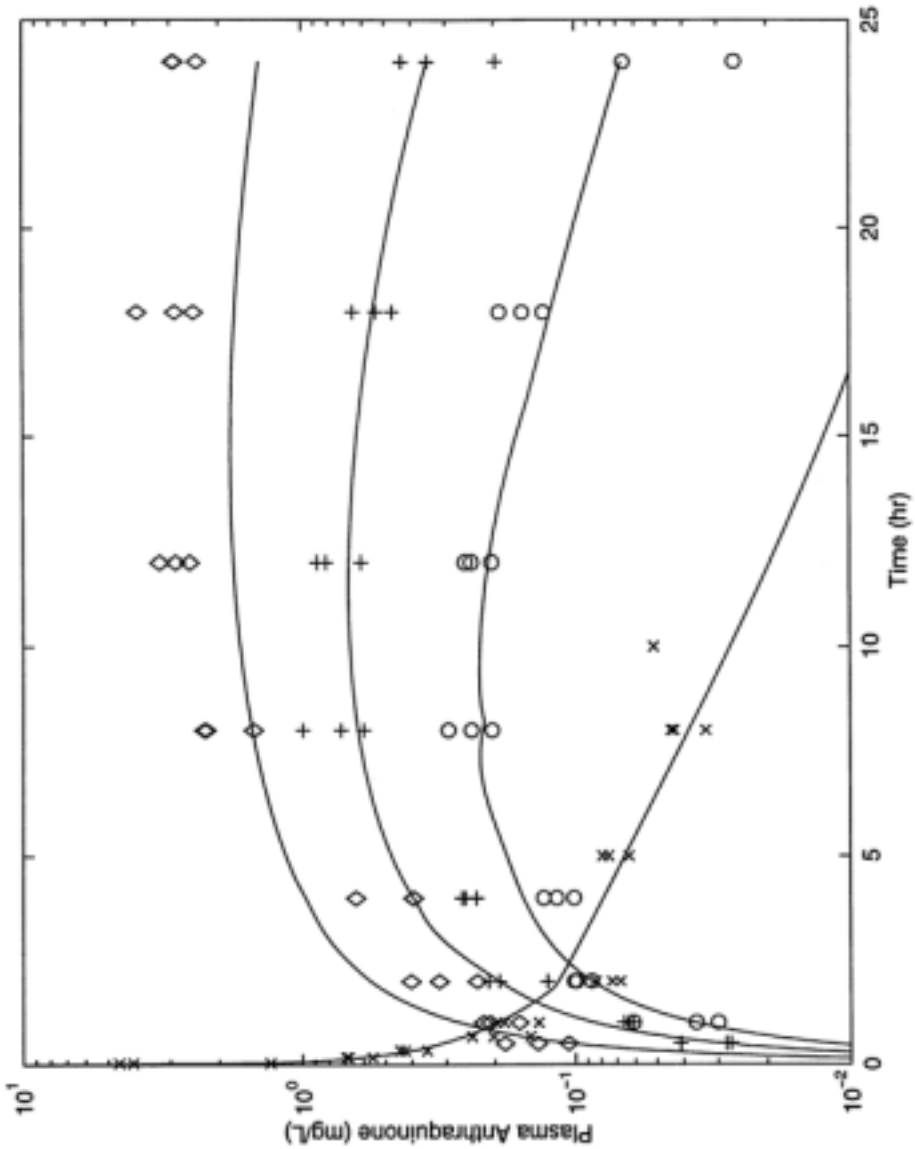


FIGURE 8
Plasma Anthraquinone Concentrations in Female Rats after a Single Intravenous or Gavage Dose. The solid lines represent the fit of these data to the physiologically based pharmacokinetic model.

TABLE 19
Optimal Parameter Values for the Physiologically Based Pharmacokinetic Model Based on Plasma Time-Course Data from the Rat Single Intravenous and Gavage Dose Studies of Anthraquinone

	Parameter						
	Absorption		Metabolic and Biliary Elimination			Capillary Permeability	
	V_{abs} (mmol/L per hour)	K_{abs} (mM)	V_{max} (mmol/L per hour)	K_m (mM)	k_{bile} (mmol/L per hour)	$Perm_{discon}$[a]	$Perm_{cont}$[b]
Male	0.44	8.4	77	5.6	0.65	0.71	0.15
Female	0.51	9.3	26	0.0047	0.65	0.47	0.17

	Tissue/Blood Partition Coefficient				
	Fat	Liver	Kidney	Slowly Perfused Tissues	Rapidly Perfused Tissues
Male	120	13	8.6	4.3	8.6
Female	130	14	9.2	4.6	9.2

[a] Liver and kidney
[b] All other organs

Based on the plasma time-course data from the intravenous and gavage studies, the optimized PBPK model was used to predict plasma concentrations of anthraquinone in rats after exposure to anthraquinone in feed for 8 days and 3, 6, 12, and 18 months. For this extrapolation of plasma dosimetry from single-dose studies to chronic exposure, animal body weights were modeled to change with age in accordance with the data shown in Table 19. Changes in cardiac output, organ volumes, and organ blood perfusion rates at 18 months were adjusted to body weight as indicated in Table I12. For the most part, feed consumption remained nearly constant throughout the 18-month exposure (Tables K1 and K2); however, body weights of exposed rats increased (Tables 5 and 6). Exposure to anthraquinone in feed was assumed to be constant from 7 pm to 3 am each day. Based on the PBPK model that was optimized to the plasma time-course data from the gavage exposure, estimates of plasma concentrations of anthraquinone from exposure in feed were overpredicted in all exposed groups. For the feed studies, the log-weighted sum of squared errors for the fit of the model simulations to the plasma anthraquinone data was 15.0 for males and 21.7 for females.

One potential source of error in this simulation is that the oral absorption parameters derived from the gavage study may not be adequate to characterize the oral absorption from the feed studies. For example, the dosing vehicle (0.2% aqueous methylcellulose and 0.1% Tween 80) used in the single-dose gavage study may affect the absorption kinetics of anthraquinone. Therefore, the absorption parameters in the anthraquinone PBPK model were reoptimized against the plasma time-course data obtained after 8 days of exposure in feed. In the reoptimized model, the absorption rate constants, V_{abs} and K_{abs}, were 0.17 mmol/L per hour and 0.58 mM for males and 0.24 mmol/L per hour and 1.2 mM for females. The reoptimized absorption parameters provide a significantly better fit ($P < 0.025$ for males; $P < 0.005$ for females) to the experimental time-course plasma anthraquinone data from feed exposure (log-weighted sum of squared errors was 7.0 for males and 1.0 for females).

Model-predicted plasma concentrations of anthraquinone in male and female rats are shown in Figures 9 and 10. Because these graphs depict the instantaneous plasma concentrations of anthraquinone, the upper and lower limits of each curve's daily excursion reflect the daily maximum and minimum plasma concentrations of anthraquinone for each exposed group throughout 18 months. The rise in plasma anthraquinone during the first month of the study reflects the accumulation of absorbed anthraquinone with continuous daily exposure. During this time, anthraquinone is being absorbed faster than it is being eliminated. As noted above, the PBPK model indicates that anthraquinone is slowly and incompletely absorbed, is distributed slowly to tissues by a diffusion-limited transport process, is stored in fatty tissues, and is slowly metabolized.

The plasma concentration of anthraquinone can serve as an internal dosimeter consequent to exposure in feed. For the current rat feed studies, the model-based plasma anthraquinone concentrations are shown in Figures 9 and 10. The estimated mean, minimum, and maximum daily concentrations of anthraquinone in plasma, liver, and kidney for each exposed group at 12 months are listed in Table 20. Because the concentration of anthraquinone in the kidney is proportional to the plasma concentration in females but not in males,

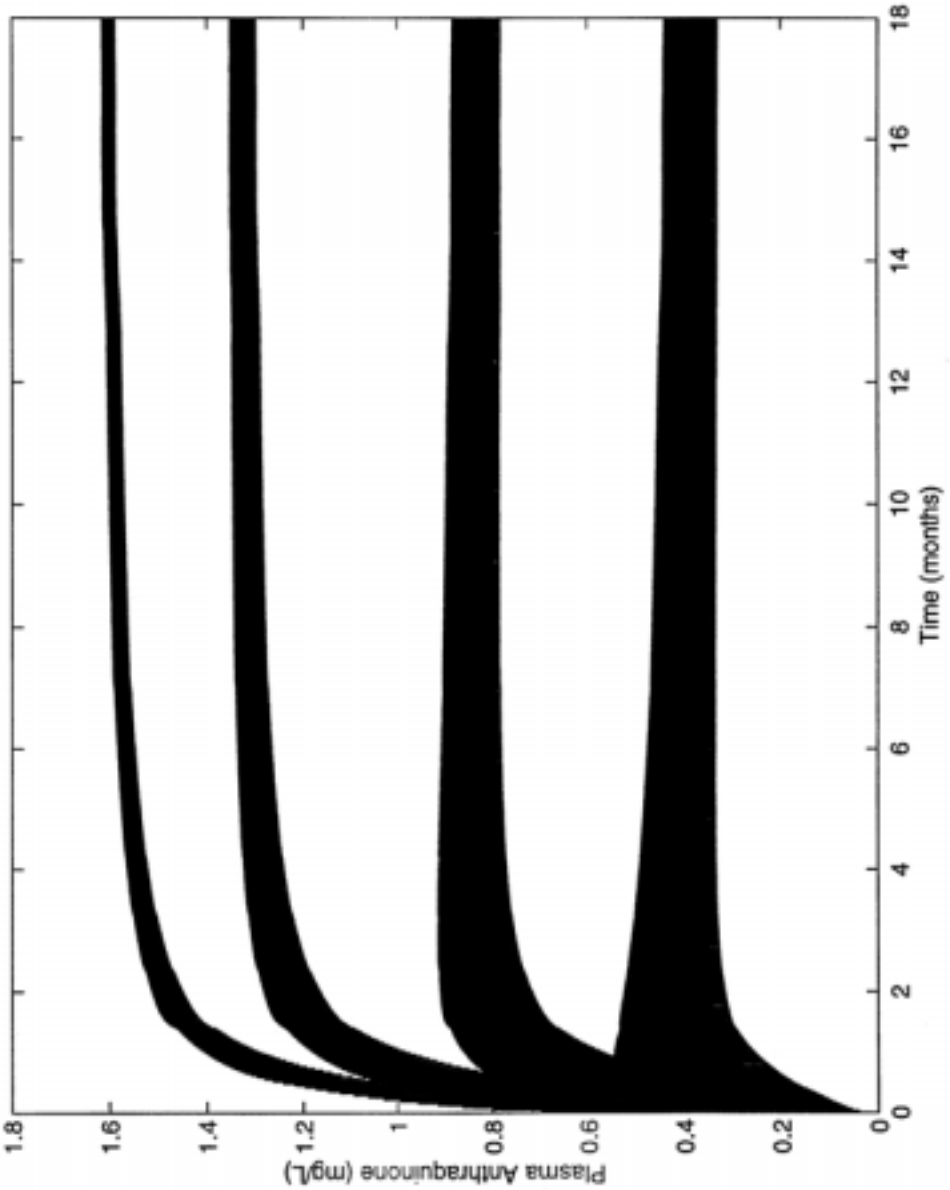


FIGURE 9
Model-Predicted Plasma Anthraquinone Concentrations in Male Rats Exposed to Anthraquinone in Feed *ad libitum* using Optimized Absorption Parameters

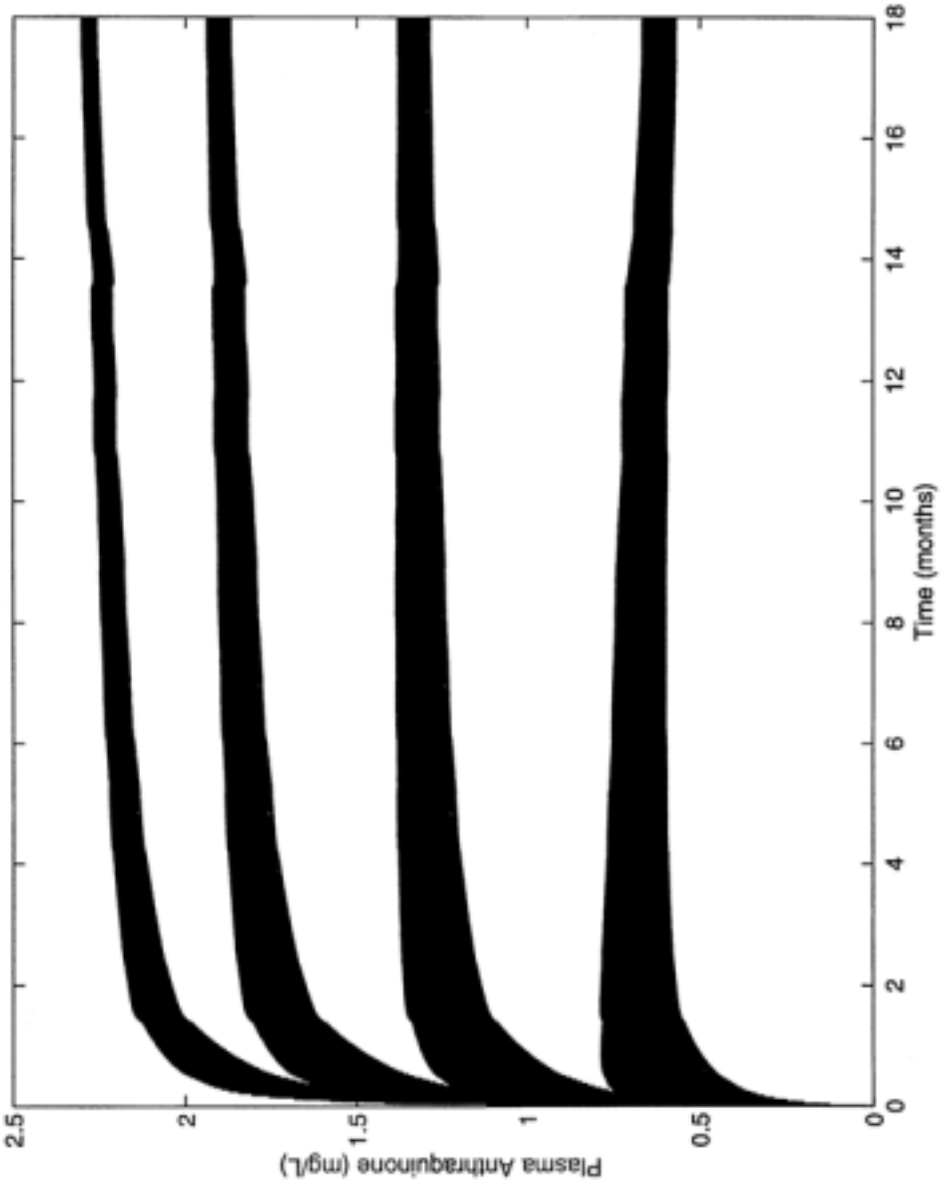


FIGURE 10
Model-Predicted Plasma Anthraquinone Concentrations in Female Rats Exposed to Anthraquinone in Feed *ad libitum* using Optimized Absorption Parameters

TABLE 20
Estimated Tissue Concentrations of Anthraquinone in Rats after 12 Months of Exposure to Anthraquinone in Feed

	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Male				
Plasma (mg/L)				
Mean	0.16	0.34	0.89	2.32
Minimum	0.13	0.28	0.79	2.19
Maximum	0.18	0.38	0.98	2.43
Liver (μg/g)				
Mean	1.37	2.91	7.76	20.16
Minimum	1.13	2.45	6.87	19.02
Maximum	1.58	3.31	8.49	21.08
Kidney (μg/g)				
Mean	0.50	1.42	6.43	22.07
Minimum	0.39	1.09	5.37	20.60
Maximum	0.59	1.73	7.31	23.25
Female				
Plasma (mg/L)				
Mean	0.66	1.33	1.87	2.24
Minimum	0.59	1.26	1.82	2.20
Maximum	0.72	1.38	1.91	2.27
Liver (μg/g)				
Mean	6.12	12.25	17.24	20.64
Minimum	5.48	11.61	16.74	20.31
Maximum	6.64	12.76	17.63	20.89
Kidney (μg/g)				
Mean	7.82	17.74	25.82	31.32
Minimum	6.82	16.72	25.01	30.79
Maximum	8.65	18.57	26.46	31.73

model-based plasma anthraquinone concentrations may serve as a surrogate dosimeter for evaluating kidney neoplasm exposure response only in females. The model-based estimates of organ concentrations of anthraquinone are dependent on the permeability, blood perfusion rate, and metabolic activity of each organ.

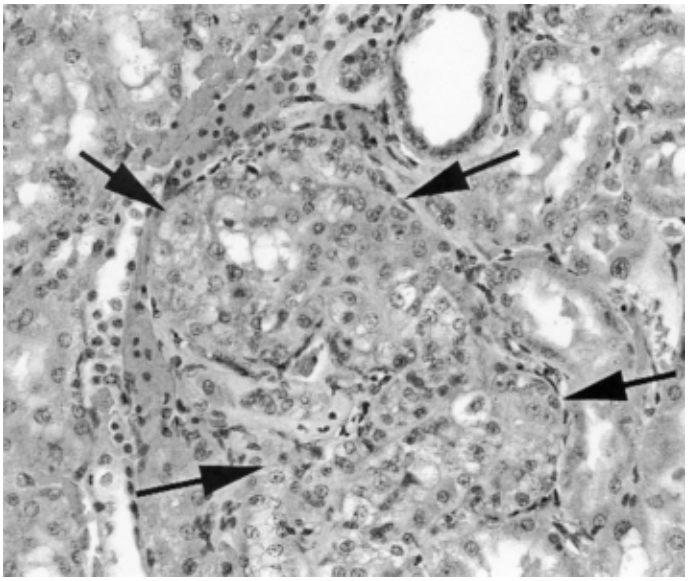


Plate 1
Renal tubule hyperplasia (arrows) in a female rat exposed to 3,750 ppm anthraquinone for 2 years. H&E; 220x

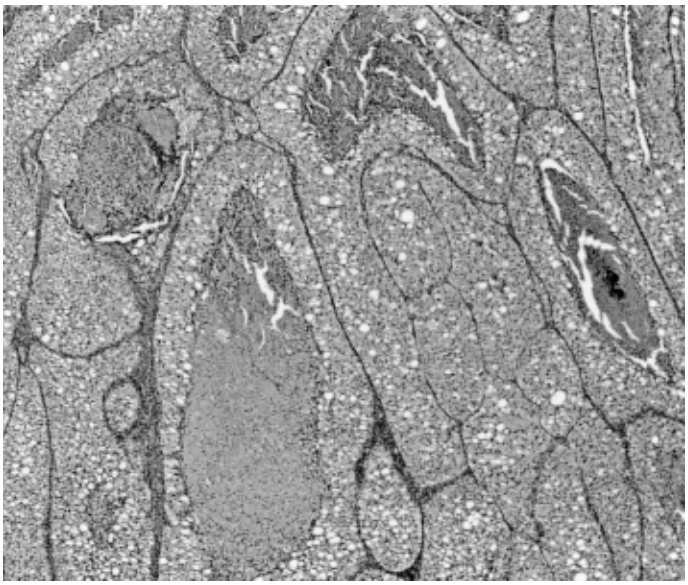


Plate 2
Renal tubule carcinoma in the kidney in a female rat exposed to 3,750 ppm anthraquinone for 2 years. H&E; 50x

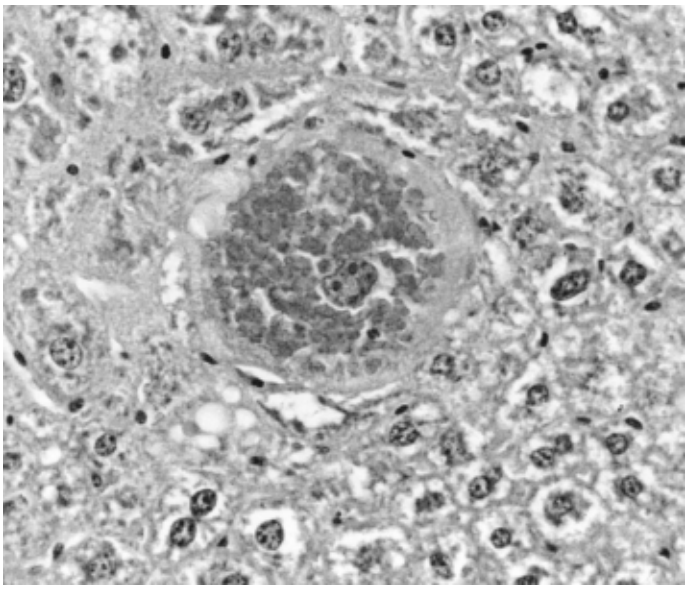


Plate 3
Liver from a male mouse exposed to 7,500 ppm anthraquinone for 2 years. An enlarged hepatocyte has phagocytized erythrocytes which surround the nucleus and have displaced the hepatocyte cytoplasm. H&E; 350x

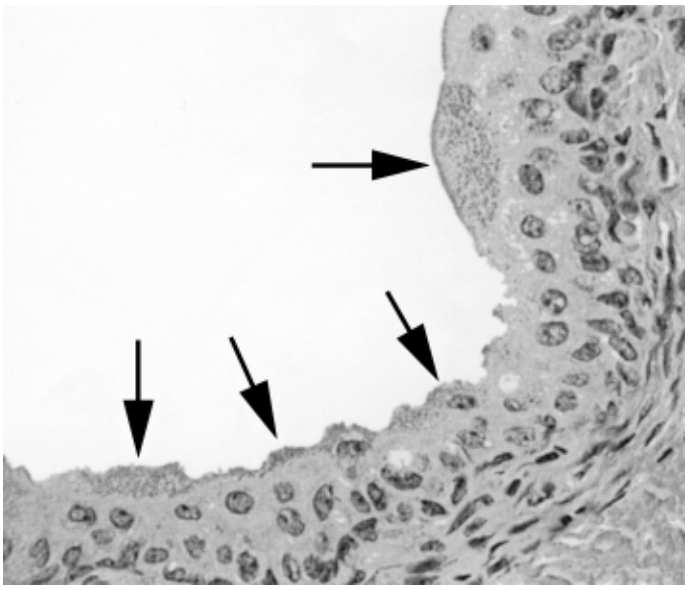


Plate 4
Urinary bladder from a female mouse exposed to 7,500 ppm anthraquinone for 2 years. Brightly eosinophilic inclusions (arrows) are present in the apical cytoplasm of luminal transitional epithelium. H&E; 460x

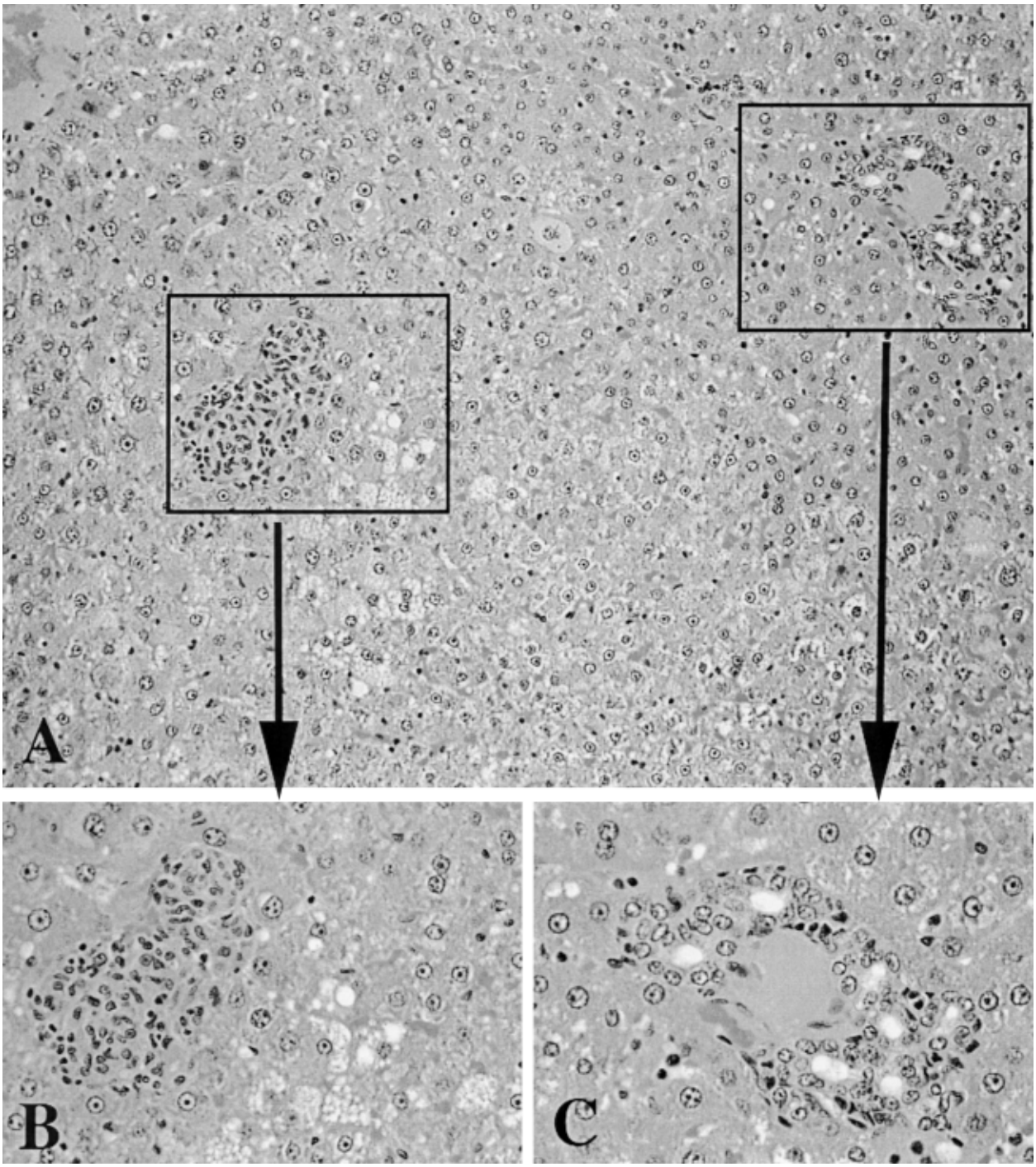


PLATE 5

Liver from a male rat exposed to 3,750 ppm anthraquinone for 2 years. An area of minimal inflammation and hepatocyte cytoplasmic vacuolization is seen in the rectangle on left and mild bile duct hyperplasia in the rectangle on the right of Figure A. Higher magnifications of these respective areas are shown in Figures B & C. Subtle hepatocyte hypertrophy is present around the central vein (upper left of Figure A). H&E; Figure A - 150x; Figures B & C - 225x

DISCUSSION AND CONCLUSIONS

Anthracene-9,10-dione, commonly referred to as anthraquinone, is present in a large number of biologically active compounds, both natural and man-made, to which there is extensive human exposure. Naturally occurring anthraquinones are widely distributed in the plant kingdom where they occur as unconjugated aglycones or as O-glycosides. Several drugs are based on the anthraquinone ring system, including the anthracycline glycosides doxorubricin and daunorubricin, which are used extensively in cancer chemotherapy as well as newer chemotherapeutic agents such as mitoxantrone. A variety of amino-, nitro-, and halogen-substituted anthraquinones are used in the manufacture of dyes.

The primary industrial use of anthraquinone is as an intermediate in the synthesis of numerous widely used dyes. It is also used in wood pulping for paper manufacture and may be found in wastewater from pulp and paper plants. Because anthraquinone is the parent for a class of compounds to which there is significant human exposure and because of the potential for exposure to anthraquinone itself, it was selected for in-depth evaluation. In addition to the 14-week and 2-year studies presented in this report, a special 32-day study was conducted after completion of the 2-year study in an attempt to provide more detailed information about the effect of anthraquinone exposure on specific biochemical endpoints. The 32-day study was conducted with the concentrations used in the 2-year study to characterize the response to the exposure concentrations (Battelle, 1999).

Anthraquinone administered in feed for 14 weeks at concentrations up to 30,000 ppm was associated with reduced mean body weights and body weight gains in female rats and produced a number of treatment-related effects in males and females. Hyaline droplets were present in the kidney of all exposed rats in the 14-week study. The droplets were positive for Mallory-Heidenhain staining in males and females, indicating that they contained protein; however, their morphology and appearance after staining were different between males and females. The presence of increased concentrations of α2u-globulin in the

kidneys of male rats from all exposed groups was confirmed by ELISA (females were not examined). The droplets in female rat kidneys were PAS positive, while the droplets in males were not. At the end of the 14-week study, the severities of nephropathy were increased in exposed male rats, and the incidences and severities of nephropathy were increased in exposed female rats. Increased urea nitrogen and creatinine concentrations in serum, normalized protein and glucose concentrations in urine, and urinary aspartate aminotransferase and *N*-acetyl-β-D-glucosaminidase activities were consistent with impaired renal function and the increased incidences and/or severities of nephropathy.

A transient erythrocytosis was observed on day 4 and was consistent with a physiologic response to dehydration, possibly associated with decreased feed consumption during the first week of the study. The increased concentrations of total serum protein and serum albumin on day 22 and at study termination were also consistent with an altered hydration status. By the third week of the study, there was evidence of a minimal to mild, macrocytic, responsive anemia, the severity of which may have been masked somewhat by dehydration. There was also evidence of an erythropoietic response to the anemia, demonstrated by increased mean cell volume and reticulocyte counts and increased incidences of bone marrow hyperplasia and congestion, hematopoietic cell proliferation, and pigmentation (positive for Perl's iron stain) in the spleen. A minimal to mild, macrocytic, responsive anemia also occurred in mice exposed to anthraquinone for 14 weeks and was evident in all groups of exposed females. As was the case with rats, an erythropoietic response and pigmentation were evident in the spleen. These results suggest an increased rate of erythrocyte turnover associated with anthraquinone exposure in both rats and mice.

During the first week of the 14-week rat study, there were treatment-related increases in alanine aminotransferase and sorbitol dehydrogenase activities and bile salt concentrations. However, this ameliorated with time, and only increases in sorbitol dehydrogenase activity were observed in 30,000 ppm males and exposed groups of females at the end of the study. While increases in alanine aminotransferase and sorbitol dehydrogenase activities are used to indicate hepatocellular injury or increased cell membrane

permeability, increases in bile salt concentrations are typically a marker of cholestasis. Thus, the decreases by day 22 in alkaline phosphatase activity, another marker of cholestasis, would appear to be incongruous with the increase in bile salt concentrations. However, serum bile salt concentrations can also be affected by altered enterohepatic circulation and impaired hepatic function, and noncholestatic liver injury can elevate circulating bile acid concentrations (Hofmann, 1988). In contrast, serum alkaline phosphatase activity increases minimally in response to hepatocellular damage (Hoffmann *et al.*, 1989). Circulating alkaline phosphatase in a normal rat is primarily of intestinal and bone origin (Righetti and Kaplan, 1971), and fasting or feed restriction causes decreases in serum alkaline phosphatase activity (Jenkins and Robinson, 1975; Imai *et al.*, 1981). If rats consumed less feed, perhaps due to reduced palatability, decreases in alkaline phosphatase activity might be related to loss of the normally circulating intestinal fraction. Therefore, the increases in bile salt concentrations in the present study would be consistent with the increased alanine aminotransferase and sorbitol dehydrogenase activities and indicative of a chemical-related effect on the liver. The decreases in alkaline phosphatase activity would suggest reduced feed intake and would be supported by the decreased body weights of exposed female rats.

Hepatocellular hypertrophy was present in all groups of exposed rats in the 14-week study. The severity was minimal in the 1,875 ppm groups, increased to mild in the 3,750, 7,500, and 15,000 ppm groups, and increased to moderate in 30,000 ppm males. The relationship between hepatocellular hypertrophy and hepatocarcinogenesis in mice has been examined in detail (Butler, 1996); however, its potential role in the development of hepatocellular neoplasms in rats is not well characterized. Therefore, the 2-year rat study design included a 3,750 ppm group with 3- and 12-month interim evaluations. This was the lowest exposure concentration that produced centrilobular hypertrophy of mild severity, a grade higher than the minimal severity observed at 1,875 ppm. Centrilobular hypertrophy of mild severity was also observed at 7,500 and 15,000 ppm; however, these concentrations increased the possibility of more severe nephropathy over the period of anthraquinone exposure. The original intent was to determine whether, with continued exposure, the centrilobular hypertrophy would become more severe and potentially hepatotoxic or remain

more or less constant. At the 3- and 12-month interim evaluations, the severity of centrilobular hypertrophy remained mild, as had been observed at the end of the 14-week study. In addition, survival, mean body weights, and clinical findings in male and female rats exposed to 1,875 or 3,750 ppm were virtually the same at 3 and 12 months. Based on these results, the 3,750 ppm group was given dosed feed until the end of the study.

Exposure to anthraquinone for 2 years caused increased incidences of a number of lesions in the kidney and urinary bladder of male and female rats. Hyaline droplets were present in the kidney of 3,750 ppm male and female rats at the 3- and 12-month interim evaluations. At the end of the 2-year study, the incidences of hyaline droplet accumulation were increased in all groups of male and female rats exposed to anthraquinone; however, the incidences in females were much higher than those in males. Nephropathy was present in all male rats, including the controls, and the severities were increased in exposed groups as indicated by the increased incidences of parathyroid gland hyperplasia, fibrous osteodystrophy, and mineralization of the glandular stomach, forestomach, and lung. The incidences of nephropathy in exposed female rats were also increased; however, no increased incidences of parathyroid gland hyperplasia or other associated lesions occurred in female rats.

The incidences of renal tubule adenoma in males were increased, but only the increase in the 938 ppm group was significant. The incidences of renal tubule hyperplasia were increased in the 469, 1,875, and 3,750 ppm groups, but the increases were not significant. The incidences of renal tubule adenoma in female rats followed a positive trend and were significantly increased in the 938, 1,875, and 3,750 ppm groups. In addition, renal tubule carcinomas were present in two 469 ppm, one 1,875 ppm, and two 3,750 ppm female rats. The incidences of renal tubule hyperplasia were significantly increased in all exposed groups of females. Papillomas of the transitional epithelium of the kidney were present in two male rats that received 938 ppm and one male that received 3,750 ppm. The incidences of hyperplasia of

the transitional epithelium were significantly increased in all exposed groups of males and in 469, 938, and 3,750 ppm females.

Transitional epithelial papillomas of the urinary bladder occurred in all groups of exposed males and in 1,875 and 3,750 ppm females; the incidence in 1,875 ppm males was significantly increased, and one 3,750 ppm female had a transitional epithelial carcinoma. The incidences of hyperplasia of the transitional epithelium of the urinary bladder were marginally increased in 1,875 and 3,750 ppm females.

Renal tubule adenomas are rare in male rats and occurred with a historical control incidence of 7/902 in recent NTP feed studies; however, they are even more uncommon in female rats and have not been observed in any control female rats in feed studies in the current historical control database (NTP, 1998). Papillomas of the transitional epithelium of the kidney in male rats and the transitional epithelium of the urinary bladder in male and female rats are also uncommon neoplasms. The increased incidences of renal tubule hyperplasia and hyperplasia of the transitional epithelium of the kidney and urinary bladder, as well as the increased incidences of rare neoplasms in these tissues, are clearly associated with chemical exposure.

It is unclear whether the presence of protein droplets in the kidneys of male and female rats had any involvement in the development of renal neoplasms. No additional attempt was made to identify the protein composition of the droplets in the kidneys of female rats. Although the concentration of α2u-globulin was increased in male rat kidneys, no increases in mean labeling indices in the kidneys of males or females was determined from BrdU incorporation during the special 32-day study (Battelle, 1999), nor was renal tubule cell hyperplasia found at the 3- or 12-month interim evaluations in the 2-year study. Therefore, the overall response was not indicative of male rat-specific α2u nephropathy.

Exposure to anthraquinone for 2 years caused increased incidences of numerous nonneoplastic lesions in the liver of male and female rats. Hepatocellular adenomas were present in all groups of exposed female rats,

and the incidence in the 938 ppm group was significantly greater than the control incidence. The incidences of hepatocellular adenoma in 938, 1,875, and 3,750 ppm female rats exceeded the historical control range. The marked increases in the incidences of nonneoplastic lesions and the presence of hepatocellular adenomas in all exposed groups of female rats were consistent with an association with anthraquinone exposure. Hepatocellular adenomas were also present in all exposed groups of male rats, and hepatocellular carcinoma was present in one male that received 1,875 ppm and one that received 3,750 ppm. The incidences of hepatocellular neoplasms were not significantly increased in any exposed group and were at the upper end of the historical control range. However, because of the increased incidences of nonneoplastic lesions, the presence of hepatocellular carcinomas in the two highest exposed groups, and the increased incidences of hepatocellular adenomas in females, the occurrence of hepatocellular neoplasms was considered an uncertain finding.

Exposure to anthraquinone for 2 years produced a marked neoplastic response in the liver of male and female mice. The response in males was particularly noteworthy and involved significant, exposure concentration-related increases in the incidences of hepatocellular carcinoma and hepatoblastoma in addition to hepatocellular adenoma. The reduced survival of 7,500 ppm male mice was due to morbidity and mortality associated with the presence of hepatocellular neoplasms.

Both the neoplastic and nonneoplastic responses observed in the liver and kidney of exposed male and female rats were characterized by examples of nonuniform dose response. This included the incidences of renal tubule hyperplasia in males and females, renal tubule adenomas in males, most nonneoplastic lesions in the liver of males and females, and hepatocellular adenomas in females. In addition, female rats were more responsive to anthraquinone exposure than male rats.

At least part of the explanation for this anomalous response may be related to the relative internal dose of anthraquinone. At 3, 6, 12, and 18 months and at each exposure concentration, the concentration of

anthraquinone in plasma in female rats was approximately twice the concentration in male rats. This indicates that female rats experienced approximately twice the exposure concentration of males. In addition, at most time points the increase in plasma concentration was not linear with exposure concentrations between 938 and 3,750 ppm in either males or females.

In an attempt to better characterize tissue concentrations of anthraquinone, a pharmacokinetic model was developed using single intravenous injection, single gavage dosing, and chronic feed toxicokinetic data from the current studies and literature data. For rats, optimal fit of the data was obtained with a model in which anthraquinone was absorbed slowly from the gastrointestinal tract via a saturable process, distributed slowly to tissues by a diffusion-limited transport process, and slowly metabolized. Tissue concentrations calculated from the optimized model indicated that plasma, liver, and kidney anthraquinone concentrations in females were three to fivefold greater than those for males except at 3,750 ppm. The model suggests that the difference is the result of slower metabolism in females. However, because knowledge of the metabolism of anthraquinone is incomplete, no metabolic data were included during the course of these studies. The model also indicates that the increases in anthraquinone tissue concentrations are not linear between the 938 and 3,750 ppm concentrations.

Exposure to anthraquinone produced similar responses in the liver of rats and mice including significant increases in liver weights and incidences of centrilobular hypertrophy characterized by increased amounts of eosinophilic cytoplasm. This type of response is characteristic of the induction of cellular biosynthetic machinery, and the results of the special 32-day study are consistent with this interpretation (Battelle, 1999). After 8 days of exposure to 469, 938, or 3,750 ppm, there was a slight increase (two to threefold above control) in cytochrome P4501A1 activity (ethoxyresorufin-*O*-dealkylase activity), but a marked, exposure concentration-related increase (80-fold over control for males, 40-fold over control for females) in cytochrome P4502B1 activity (pentoxyresorufin-*O*-dealkylase activity) in microsomes prepared from the livers of male and female rats. Induction approximately two to threefold greater in males than in females,

which correlated with the greater severity of hepatocellular hypertrophy in males than in females. No significant increase or decrease in mean labeling index determined from BrdU incorporation into hepatocyte nuclei was found in rats. Although this represents a single window of time after a relatively short exposure period, it is consistent with the results of the 14-week study and the 3- and 12-month interim evaluations during the 2-year study, all of which reveal no histologic evidence of an increased proliferative response in the liver of exposed rats.

Induction of cytochrome P4502B1 activity is considered an indicator of phenobarbital-type induction in rodents and is characterized by increases in the expression of numerous other genes, many coding for other enzymes involved in detoxication (Nims and Lubet, 1996). However, the toxicokinetic data and modeling suggest that anthraquinone is metabolized slowly and does not induce enzymes that increase the rate of its metabolism.

The incidences of mononuclear cell leukemia were markedly reduced in exposed male and female rats. Although splenic toxicity is often correlated with reduced incidences of mononuclear cell leukemia (Elwell *et al*. 1996), it is unlikely that the mild nature of the lesions that occurred in the spleen in the current study could account for the dramatic decrease in incidences. This suggests that the reduction was due to a direct effect of anthraquinone or its metabolite(s) on the development of mononuclear cell leukemia. Similar decreases have been observed in the 2-year studies of 1-amino-2,4-dibromoanthraquinone and emodin (NTP, 1996, 2001).

Thyroid gland follicular cell adenomas were present in two male mice each in the 2,500 and 7,500 ppm groups, and the incidences of follicular cell hyperplasia were significantly increased in these groups. Follicular cell adenomas were present in all groups of exposed female mice, and the incidences of follicular cell carcinoma and adenoma or carcinoma (combined) in the 7,500 ppm females exceeded the historical control ranges. The presence of follicular cell adenomas and increased hyperplasia in 7,500 ppm males and

increased incidences of follicular cell adenoma or carcinoma (combined) in 7,500 ppm females suggest an association with anthraquinone exposure, and the neoplasms were considered uncertain findings.

Anthraquinone and substituted anthraquinones readily form stable complexes with DNA and other double-stranded polynucleotides by intercalation (Islam *et al.*, 1985; Tanious *et al.*, 1992), a process that involves sliding of the planar anthraquinone ring between adjacent stacked base pairs (Neidle and Abraham, 1984). In addition, anthraquinone and most substituted anthraquinones are mutagens. Therefore, it is not surprising that many anthraquinones have been carcinogenic in long-term animal studies. The substituents present in the anthraquinone ring have a major impact on the carcinogenic response as well as on the target organs involved (Table 21). Most of the compounds shown in Table 21 are carcinogens and carry halogen, amino, or nitro substitutions. Although the liver is a common site, carcinogenic responses have also occurred in the gastrointestinal tract in rats and mice and the kidney and urinary bladder in rats. In the NTP database (NTP, 1998), 1-amino-2,4-dibromoanthraquinone is the only compound administered in feed other than anthraquinone that produced renal neoplasms in both male and female rats.

In contrast, emodin (1,3,8-trihydroxy-6-methylanthraquinone), a trihydroxy-substituted anthraquinone administered in feed, produced only equivocal responses in female rats and male mice (NTP, 2001), while 1,8-dihydroxyanthraquinone and 1-hydroxyanthraquinone administered in feed targeted the large intestine. Thus, while the anthraquinone ring endows this class of compounds with the capacity to interact with DNA via intercalation, the substituents present on the ring determine the ultimate mutagenic and carcinogenic activity of individual anthraquinones. Undoubtedly, the influence of substituents on metabolism is a major factor influencing carcinogenicity and mutagenicity.

TABLE 21
Exposure Concentrations in the NCI/NTP 2-Year Feed Studies of Anthraquinone Derivatives

Anthraquinone Derivative	Low Dose (ppm)	High Dose (ppm)	Carcinogenic Response	Reference
Rats				
Male				
Anthraquinone	469	3,750	liver[b], kidney, urinary bladder	
2-Aminoanthraquinone[a]	3,500	6,900	liver	TR 144; NCI, 1978a
1-Amino-2,4-dibromoanthraquinone	2,000	10,000	liver, large intestine, kidney, urinary bladder	TR 383; NTP, 1996
1-Amino-2-methylanthraquinone[a]	1,000	2,000	liver, kidney	TR 111; NCI, 1978b
Emodin	280	2,500		TR 493; NTP, 2001
2-Methyl-1-nitroanthraquinone	600	1,200	liver, skin	TR 29; NCI, 1978c
1,4,5,8-Tetraaminoanthraquinone	1,250	5,000	urinary bladder, pancreas	TR 299; NTP, 1986
Female				
Anthraquinone	469	3,750	liver, kidney, urinary bladder	
2-Aminoanthraquinone[c]	2,000			TR 144; NCI, 1978a
1-Amino-2,4-dibromoanthraquinone	2,000	10,000	liver, large intestine, kidney, urinary bladder	TR 383; NTP, 1996
1-Amino-2-methylanthraquinone	1,000	2,000	liver	TR 111; NCI, 1978b
Emodin	280	2,500	Zymbal's gland[b]	TR 493; NTP, 2001
2-Methyl-1-nitroanthraquinone	600	1,200	skin	TR 29; NCI, 1978c
1,4,5,8-Tetraaminoanthraquinone	1,250	5,000	urinary bladder	TR 299; NTP, 1986
Mice				
Male				
Anthraquinone	833	7,500	liver, thyroid gland[b]	
2-Aminoanthraquinone	5,000	10,000	liver	TR 144; NCI,1978a
1-Amino-2,4-dibromoanthraquinone	10,000	20,000	liver, forestomach, lung	TR 383; NTP, 1996
1-Amino-2-methylanthraquinone	600	—[d]		TR 111; NCI, 1978b
Emodin	160	625	kidney[b]	TR 493; NTP, 2001
2-Methyl-1-nitroanthraquinone	300	600	hemangiosarcoma	TR 29; NCI, 1978c
1,4,5,8-Tetraaminoanthraquinone	600	2,500	liver[b], lung[b]	TR 299; NTP, 1986
Female				
Anthraquinone	833	7,500	liver, thyroid gland[b]	
2-Aminoanthraquinone	5,000	10,000	liver, lymphoma	TR 144; NCI, 1978a
1-Amino-2,4-dibromoanthraquinone	10,000	20,000	liver, forestomach, lung	TR 383; NTP, 1996
1-Amino-2-methylanthraquinone	600	—[d]	liver	TR 111; NCI, 1978b
Emodin	312	1,250		TR 493; NTP, 2001
2-Methyl-1-nitroanthraquinone	300	600	hemangiosarcoma	TR 29; NCI, 1978c
1,4,5,8-Tetraaminoanthraquinone	600	2,500		TR 299; NTP, 1986

[a] Exposure concentrations in this study were time-weighted averages.
[b] *Equivocal evidence* for these organs
[c] Inadequate study
[d] Two dosage regimens were used, but the time-weighted average concentrations were the same.

There are three methods used for the commercial synthesis of anthraquinone: a Friedel-Crafts reaction between benzene and phthalic anhydride, a Diels-Alder synthesis from 1,4-naphthoquinone and 1,3-butadiene, and the oxidation of anthracene in nitric acid. Anthraquinone synthesized by this latter method could conceivably contain small quantities of nitroanthracene isomers in the final purified material. The sample of anthraquinone used for the 2-year bioassays presented in this Technical Report was purchased as 99.9% pure material. The identity and purity of this sample was confirmed during the preliminary analytical chemistry studies. At the time the study was started, the 0.1% contaminant was not identified; however, it has since been confirmed that the anthraquinone sample used for the 2-year studies was prepared by oxidation of anthracene in nitric acid and that the 0.1% contaminant is 9-nitroanthracene.

Butterworth *et al.* (2001) have shown that anthraquinone synthesized by either the Friedel-Crafts or Diels-Alder methods is not mutagenic in standard bacterial mutagenicity assays. However, a sample of the anthraquinone used in the NTP 2-year bioassays presented in this Technical Report, containing 9-nitroanthracene as the major contaminant, was mutagenic under the same conditions in the absence of metabolic activation; in the presence of S9, the mutagenic activity was reduced or eliminated, suggesting that the mutagenic component was direct acting. Purification of the NTP anthraquinone sample to remove the 9-nitroanthracene contaminant resulted in loss of mutagenic activity. Based on these results, Butterworth *et al.* (2001) assumed that all mutagenic activity present in the NTP anthraquinone sample was attributable to the 9-nitroanthracene contaminant. However, they did not evaluate the mutagenicity of a bona fide sample of 9-nitroanthracene.

In their analysis, Butterworth *et al.* (2001) attributed the carcinogenic activity observed in the current 2-year studies entirely to the mutagenic contaminant even though the carcinogenicity of 9-nitroanthracene has not been evaluated. They did not consider the possible involvement of anthraquinone metabolites. However, preliminary studies conducted by the NTP indicated that

anthraquinone is metabolized extensively, with the two primary urinary metabolites being 1- and 2-hydroxyanthraquinone. 1-Hydroxyanthraquinone administered in the diet to male ACI/N rats induced tumors of the liver, stomach, and large intestine (Mori *et al.*, 1990), and 2-hydroxyanthraquinone has been reported to be a bacterial mutagen (Tikkanen *et al.*, 1983).

Based on the questionable mutagenicity of anthraquinone, the presence of contaminating 9-nitroanthracene, and formation of the potentially carcinogenic metabolites 1- and 2-hydroxyanthraquinone, it was considered important to the proper interpretation of the 2-year anthraquinone carcinogenicity studies to reevaluate the mutagenicity of samples of these compounds of known purity, following identical protocols, and in the same laboratory.

The mutagenicity of anthraquinone has been somewhat controversial. In studies reported by the NTP, anthraquinone was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 in the presence and absence of S9 activation (Zeiger *et al.*, 1988); the material used for these studies was purchased from Aldrich Chemical Co. and was 97% pure, but no further purification or identification of contaminants was conducted. Only one other positive Ames test result has been published for anthraquinone, but several papers have reported that anthraquinone was not mutagenic in the Ames test. In the present study, we have confirmed the nonmutagenicity of pure anthraquinone, suggesting that the positive results reported previously were due to the presence of mutagenic contaminant(s). Interestingly, although 1-hydroxyanthraquinone has been reported to be a carcinogen in rats (Mori *et al.*, 1990), it was not mutagenic in any of the assays. However, 2-hydroxyanthraquinone was mutagenic in TA98 in the absence of S9 and gave an equivocal response in the presence of S9.

Fu *et al.* (1985a) examined the mutagenicity of 9-nitroanthracene and the metabolism of 9-nitroanthracene by microsomes isolated from the livers of uninduced rats. Four metabolites were identified: the *trans*-1,2 and *trans*-3,4 dihydrodiols of 9-nitroanthracene, the 1,2,3,4-tetrahydrotetrol

of 9-nitroanthracene, and anthraquinone. The dihydrodiols are likely formed from 1,2- and 3,4-epoxide intermediates, and the tetrol from one of the dihydrodiols. 9-Nitroanthracene was not reduced to 9-aminoanthracene, even under hypoxic conditions where competing oxidative reactions should be minimized. Moreover, 9-nitroanthracene as well as its metabolites were judged by Fu *et al.* (1985a) to be at best weakly mutagenic in TA98 or TA100 in the presence or absence of S9.

In a later study, Fu *et al.* (1986) examined the microsomal metabolism of both 2- and 9-nitroanthracene, in this case using microsomes prepared from the livers of rats induced with 3-methylcholanthrene (3-MC). 9-Nitroanthracene was converted to the same metabolites by microsomes from 3-MC induced rats as previously reported for microsomes from uninduced rats (Fu *et al.*, 1985a). Moreover, 9-nitroanthracene was not nitroreduced under hypoxic conditions. In contrast, 2-nitroanthracene was converted to 5,5- and 7,8-dihydrodiols, 6-keto- and 7-keto-tetrahydrodiols, 2-nitro-9,10-anthraquinone, and a tetrahydrotetrol in the presence of oxygen. Under hypoxic conditions, 2-nitroanthracene was readily reduced to 2-aminoanthracene. Moreover, 2-nitroanthracene was a potent direct-acting mutagen in TA98.

Aromatic nitro compounds in which the nitro group is forced out of co-planarity with the aromatic ring system because of steric hindrance with neighboring groups apparently are not efficiently reduced to amines by bacterial nitroreductases and, therefore, are not usually direct-acting mutagens (Fu *et al.*, 1985b; Jung *et al.*, 1991). There are several examples where isomeric pairs of nitroarenes have been evaluated and this relation has been found to hold (Fu *et al.*, 1985b, 1997; Jung *et al.*, 1991). The nitro group orientation in 9-nitroanthracene is approximately 90 degrees to the anthracene ring, whereas that of 2-nitroanthracene is coplanar with the anthracene ring. Therefore, Fu *et al.* (1986) attributed the weak mutagenicity of 9-nitroanthracene to the non-coplanar orientation of the nitro group.

9-Nitroanthracene was mutagenic in both TA98 and TA100 in the presence and absence of S9. Zeiger *et al.* (1988) also observed positive results for 9-nitroanthracene in *Salmonella*; however, the purity of the material was not confirmed. Zeiger *et al.* (1988) used 30% rat and hamster liver S9 preparations for metabolic activation, compared to the 10% concentration used in the most recent tests (Appendix E), and doses of test chemical were notably lower in the 97% pure anthraquinone study ranging from 10 to 1,000 μg/plate compared to the 100 to 6,667 μg/plate in the 9-nitroanthracene study. Fu *et al.* (1985b) used lower doses ranging from 0.6 μg to 35 μg/plate and used a plate incorporation assay rather than the preincubation assay used in the present study. Because of these differences, it is difficult to directly compare the results observed in the present study with those of Fu *et al.* (1985b) or Zeiger *et al*. (1988).

The identification and quantitation of metabolites present in the urine of F344 rats exposed to anthraquinone manufactured by oxidation in nitric acid, by Diels-Alder synthesis, or by Friedel-Crafts synthesis are summarized in Table 22. 2-Hydroxyanthraquinone is the major anthraquinone metabolite present in urine regardless of the method of anthraquinone synthesis, with lesser amounts of 1-hydroxyanthraquinone also present. The identification of 2-hydroxyanthraquinone as a major urinary metabolite of anthraquinone is in agreement with results reported previously by Sato *et al.* (1956) and is consistent with the strong induction of CYP2B in the liver of rats exposed to anthraquinone reported in the present study.

Table 22 also shows the amount of 1- and 2-hydroxyanthraquinone excreted in urine over the 24-hour collection period. It is interesting to compare these quantities with the amount of 9-nitroanthracene that would be ingested over a 24-hour period, using feed consumption data and assuming 100% absorption and bioavailability of 9-nitroanthracene present in the anthraquinone feed mix. These data are shown in Table 22 and indicate that the animals would be exposed to a substantially greater amount of 2-hydroxyanthraquinone than 9-nitroanthracene over a typical 24-hour period. Because it

TABLE 22
Quantitation of 1- and 2-Hydroxyanthraquinone and 9-Nitroanthracene in Rat Urine

Anthraquinone Sample	Concentration (μg/mL)	Average Urine Volume Collected (mL)	Total Eliminated (μg/24 hours)
1-Hydroxyanthraquinone			
Control	0	5.6 ± 2.6	0
Nitric acid oxidation	3.688	7.1 ± 2.9	26.18
Diels-Alder synthesis, vendor K	5.933	9.3 ± 1.6	55.18
Diels-Alder synthesis, vendor E	8.305	8.0 ± 2.1	66.44
Friedel-Crafts synthesis, vendor E	5.789	9.3 ± 1.2	53.84
2-Hydroxyanthraquinone			
Control	0	5.6 ± 2.6	0
Nitric acid oxidation	163.8	7.1 ± 2.9	1,162.98
Diels-Alder synthesis, vendor K	311.1	9.3 ± 1.6	2,893.23
Diels-Alder synthesis, vendor E	278.1	8.0 ± 2.1	2,224.80
Friedel-Crafts synthesis, vendor E	293.6	9.3 ± 1.2	2,730.48
9-Nitroanthracene available for ingestion			
138[a]			

[a] The quantity of 9-nitroanthracene available for ingestion was calculated for a male rat weighing 327 g and consuming 16.7 g of feed per day containing 7,500 ppm anthraquinone and 8.3 ppm 9-nitroanthracene.

is unlikely that 9-nitroanthracene is 100% bioavailable, the actual exposure could be significantly less than indicated in Table 22.

Comparison of the revertants per microgram as an approximation of mutagenic potency for 9-nitroanthracene and 2-hydroxyanthraquinone in TA98 in the absence of S9 at 100 μg/plate (a dose used for both compounds) indicates that 2-hydroxyanthraquinone induced 2.25 revertants/μg whereas 9-nitroanthracene induced 0.315 revertants/μg (Table 21). Under these conditions, 2-hydroxyanthraquinone was approximately a sevenfold more potent, direct-acting mutagen than 9-nitroanthracene. If the comparison is made between 9-nitroanthracene in the presence of S9 (0.805 revertants/μg) and 2-hydroxyanthraquinone in the absence of S9, 2-hydroxyanthraquinone is approximately threefold more potent.

Butterworth *et al.* (2001) imply that the carcinogenicity of anthraquinone is significantly influenced by the presence of the 0.1% contaminant 9-nitroanthracene. Based on the information presented here, we conclude that if anthraquinone is carcinogenic through a mechanism involving mutagenicity, the contaminant is not a necessary component in this response. 2-Hydroxyanthraquinone is a direct acting mutagen produced *in situ*, most likely in the liver, a target organ in the present study, and it is present systemically in substantially greater quantities that are even theoretically possible for 9-nitroanthracene. Considering the low level of exposure (at the highest dose used in the 2-year rat study, males would have been exposed to 0.216 mg/kg and females to 0.24 mg/kg 9-nitroanthracene), actual bioavailability, and relative mutagenic potency, it is unlikely that the presence of 9-nitroanthracene made any significant contribution to the carcinogenic responses observed in this study.

When evaluating the impact of this information on the interpretation of the anthraquinone 2-year study, it is important to emphasize that not all mutagens are carcinogens, and not all carcinogens are mutagens. In a comprehensive review of the performance of genetic toxicity tests for 363 chemicals that have been evaluated for genetic toxicity and carcinogenicity by the NTP, Zeiger (1998) found that of 144 chemicals that were *Salmonella* mutagens, 111 (77%) were carcinogens and 33 (23%) were not carcinogens in 2-year studies. Of the 205 chemicals that were carcinogens in 2-year studies, 111 (54%) were mutagenic in *Salmonella* while 94 (46%) were not mutagenic in *Salmonella*.

In the present study, 1-nitroanthracene was mutagenic in both TA98 and TA100 in the presence and absence of rat liver S9. 2-Nitroanthracene was a very strong mutagen in both TA98 and TA100 in the presence and absence of activation, but the magnitude of the response was reduced in the presence of S9.

Comparing the response observed in the present studies of 1-, 2-, and 9-nitroanthracene, using the number of induced revertants per microgram in TA98 in the absence of S9 as an approximate measure of potency for direct-acting mutagenicity, it is apparent that 2-nitroanthracene, in which the nitro group is approximately coplanar with the anthracene ring, is a substantially more potent direct-acting mutagen than 1-nitroanthracene, which is more potent than 9-nitroanthracene (Table 23).

TABLE 23
Mutagenicity in *Salmonella typhimurium* Strain TA98

Chemical	Concentration (μg)	S9	No. of Revertants per μg[a]
1-Nitroanthracene	0.1	absent	130
2-Nitroanthracene	0.33	absent	2,422.7
9-Nitroanthracene	100	absent	0.315
		present	0.805
2-Hydroxyanthraquinone	10	absent	1.85[b]
	100	absent	2.25[b]

[a] Average of two trials except where noted. Induced revertants were calculated for each trial by subtracting zero-dose revertants from revertants observed at each dose, averaging the induced mutants, and dividing by the dose.
[b] Average of four trials

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *some evidence of carcinogenic activity** of anthraquinone in male F344/N rats based on increased incidences of renal tubule adenoma and of transitional epithelial papillomas of the kidney and urinary bladder. Hepatocellular neoplasms may have been related to exposure to anthraquinone. There was *clear evidence of carcinogenic activity* of anthraquinone in female F344/N rats based on increased incidences of renal tubule neoplasms. Increases in the incidences of urinary bladder transitional epithelial papilloma or carcinoma (combined) and of hepatocellular adenoma in female rats were also related to anthraquinone exposure. There was *clear evidence of carcinogenic activity* in male and female $B6C3F_1$ mice based on increased incidences of liver

neoplasms. Thyroid gland follicular cell neoplasms in male and female mice may have been related to anthraquinone exposure.

Exposure to anthraquinone for 2 years caused increases in the incidences of nonneoplastic lesions of the kidney, liver, spleen, and bone marrow in male and female rats, the liver, urinary bladder, and spleen in male and female mice, and the thyroid gland and kidney in male mice.

Decreased incidences of mononuclear cell leukemia in male and female rats were attributed to exposure to anthraquinone.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report from May 21, 1999, appears on page 17.

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

TABLE A1 Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Anthraquinone A-3
TABLE A2 Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone A-8
TABLE A3 Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Anthraquinone A-28
TABLE A4a Historical Incidence of Renal Tubule Neoplasms in Untreated Male F344/N Rats A-33
TABLE A4b Historical Incidence of Kidney Transitional Epithelial Papillomas in Untreated Male F344/N Rats A-33
TABLE A4c Historical Incidence of Urinary Bladder Neoplasms in Untreated Male F344/N Rats A-34
TABLE A4d Historical Incidence of Hepatocellular Neoplasms in Untreated Male F344/N Rats A-34
TABLE A4e Historical Incidence of Mononuclear Cell Leukemia in Untreated Male F344/N Rats A-35
TABLE A5 Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Anthraquinone A-36

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Disposition Summary					
Animals initially in study	60	50	50	50	60
3-Month interim evaluation	5				5
12-Month interim evaluation	5				5
Early deaths					
Moribund	21	21	16	14	17
Natural deaths	7	6	12	10	11
Survivors					
Died last week of study	1				
Terminal sacrifice	21	23	22	26	22
Animals examined microscopically	60	50	50	50	60
Systems Examined at 3 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					
12-Month Interim Evaluation					
Genital System					
Testes	(5)				(5)
Interstitial cell, adenoma, multiple	1 (20%)				1 (20%)
Systems Examined with No Neoplasms Observed					
Alimentary System					
Cardiovascular System					
Endocrine System					
General Body System					
Hematopoietic System					
Integumentary System					
Musculoskeletal System					
Nervous System					
Respiratory System					
Special Senses System					
Urinary System					

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study					
Alimentary System					
Esophagus	(50)	(50)	(50)	(50)	(49)
Fibrosarcoma, metastatic, salivary glands		1 (2%)			
Intestine small, duodenum	(50)	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)	(50)
Hepatocellular carcinoma				1 (2%)	1 (2%)
Hepatocellular adenoma	1 (2%)	3 (6%)	4 (8%)	3 (6%)	2 (4%)
Hepatocellular adenoma, multiple				1 (2%)	
Histiocytic sarcoma				1 (2%)	
Mesentery	(10)	(4)	(6)	(7)	(6)
Fibrosarcoma, metastatic, skin					1 (17%)
Oral mucosa	(1)		(2)		(1)
Squamous cell carcinoma					1 (100%)
Squamous cell papilloma	1 (100%)		1 (50%)		
Pancreas	(50)	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin					1 (2%)
Acinus, adenoma	1 (2%)				
Salivary glands	(50)	(50)	(48)	(50)	(47)
Fibrosarcoma		1 (2%)			
Schwannoma malignant		1 (2%)	1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)	(50)
Squamous cell papilloma					1 (2%)
Cardiovascular System					
Heart	(50)	(50)	(50)	(50)	(50)
Endocrine System					
Adrenal cortex	(50)	(50)	(50)	(50)	(50)
Schwannoma malignant				1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)	(50)
Pheochromocytoma malignant		1 (2%)			
Pheochromocytoma complex				1 (2%)	
Pheochromocytoma benign	6 (12%)	7 (14%)	10 (20%)	6 (12%)	10 (20%)
Bilateral, pheochromocytoma benign	2 (4%)	1 (2%)			1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		2 (4%)		
Carcinoma	1 (2%)		1 (2%)		
Parathyroid gland	(49)	(48)	(48)	(50)	(45)
Adenoma				1 (2%)	1 (2%)
Carcinoma, metastatic, thyroid gland		2 (4%)			
Pituitary gland	(50)	(50)	(49)	(50)	(50)
Pars distalis, adenoma	15 (30%)	15 (30%)	11 (22%)	11 (22%)	9 (18%)
Thyroid gland	(50)	(50)	(48)	(50)	(47)
Fibrosarcoma, metastatic, salivary glands		1 (2%)			
Schwannoma malignant, metastatic, salivary glands		1 (2%)			
Bilateral, C-cell, adenoma				1 (2%)	1 (2%)
C-cell, adenoma	2 (4%)	6 (12%)	6 (13%)	6 (12%)	3 (6%)
C-cell, carcinoma		2 (4%)			
Follicular cell, adenoma	1 (2%)		4 (8%)	1 (2%)	1 (2%)
Follicular cell, carcinoma	1 (2%)	3 (6%)		1 (2%)	

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
General Body System					
Tissue NOS			(1)		
Chemodectoma benign			1 (100%)		
Genital System					
Epididymis	(50)	(50)	(50)	(50)	(50)
Preputial gland	(50)	(49)	(50)	(49)	(50)
Adenoma	3 (6%)	8 (16%)	7 (14%)	4 (8%)	2 (4%)
Carcinoma	1 (2%)	1 (2%)	2 (4%)	5 (10%)	3 (6%)
Bilateral, adenoma		1 (2%)			
Prostate	(50)	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(49)	(50)
Testes	(50)	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	6 (12%)	5 (10%)	3 (6%)	3 (6%)	4 (8%)
Interstitial cell, adenoma, multiple	40 (80%)	41 (82%)	44 (88%)	44 (88%)	44 (88%)
Hematopoietic System					
Bone marrow	(50)	(50)	(50)	(50)	(50)
Lymph node	(8)	(3)	(1)	(1)	(2)
Mediastinal, histiocytic sarcoma				1 (100%)	
Lymph node, mandibular	(50)	(50)	(47)	(50)	(47)
Fibrosarcoma, metastatic, salivary glands		1 (2%)			
Schwannoma malignant, metastatic, salivary glands		1 (2%)			
Lymph node, mesenteric	(50)	(49)	(50)	(50)	(49)
Spleen	(50)	(50)	(50)	(50)	(50)
Fibroma				1 (2%)	
Fibrosarcoma	1 (2%)		1 (2%)		
Hemangiosarcoma		1 (2%)	1 (2%)		
Histiocytic sarcoma				1 (2%)	
Thymus	(46)	(44)	(45)	(46)	(41)
Integumentary System					
Mammary gland	(45)	(47)	(46)	(50)	(47)
Fibroadenoma	1 (2%)	5 (11%)		7 (14%)	4 (9%)
Fibroadenoma, multiple			1 (2%)	1 (2%)	
Skin	(50)	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	1 (2%)			
Keratoacanthoma		2 (4%)	3 (6%)	2 (4%)	5 (10%)
Squamous cell papilloma	1 (2%)				2 (4%)
Trichoepithelioma	1 (2%)				
Sebaceous gland, adenoma	1 (2%)				
Subcutaneous tissue, fibroma	5 (10%)	4 (8%)	1 (2%)	5 (10%)	2 (4%)
Subcutaneous tissue, fibrosarcoma			1 (2%)		1 (2%)
Subcutaneous tissue, histiocytic sarcoma				1 (2%)	
Subcutaneous tissue, lipoma			1 (2%)		
Subcutaneous tissue, melanoma malignant				3 (6%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)				

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Musculoskeletal System					
Bone	(50)	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)				
Maxilla, osteosarcoma				1 (2%)	
Tibia, chondroma	1 (2%)				
Skeletal muscle	(1)				
Osteosarcoma, metastatic, bone	1 (100%)				
Nervous System					
Brain	(50)	(50)	(50)	(50)	(50)
Astrocytoma malignant			2 (4%)		
Histiocytic sarcoma				1 (2%)	
Meningioma malignant					1 (2%)
Oligodendroglioma malignant					1 (2%)
Spinal cord	(1)				(2)
Respiratory System					
Lung	(50)	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	2 (4%)		2 (4%)	
Carcinoma, metastatic, preputial gland					1 (2%)
Chordoma, metastatic, uncertain primary site		1 (2%)			
Hepatocellular carcinoma, metastatic, liver					1 (2%)
Histiocytic sarcoma				1 (2%)	
Osteosarcoma, metastatic, bone				1 (2%)	
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)			
Nose	(50)	(50)	(50)	(50)	(50)
Respiratory epithelium, adenoma	1 (2%)				
Respiratory epithelium, papilloma				1 (2%)	
Trachea	(50)	(50)	(50)	(50)	(49)
Fibrosarcoma, metastatic, salivary glands		1 (2%)			
Special Senses System					
Zymbal's gland			(2)		
Carcinoma			2 (100%)		
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(50)
Bilateral, renal tubule, adenoma			1 (2%)		
Renal tubule, adenoma	1 (2%)	3 (6%)	8 (16%)	5 (10%)	3 (6%)
Renal tubule, oncocytoma benign			1 (2%)		
Transitional epithelium, papilloma			2 (4%)		1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)	(49)
Transitional epithelium, papilloma		1 (2%)	3 (6%)	7 (14%)	3 (6%)

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Systemic Lesions					
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)	
Leukemia mononuclear	25 (50%)	2 (4%)	1 (2%)	5 (10%)	7 (14%)
Mesothelioma malignant	4 (8%)	4 (8%)	5 (10%)	4 (8%)	
Neoplasm Summary					
Total animals with primary neoplasms[c]					
12-Month interim evaluation	1				1
2-Year study	50	50	50	50	49
Total primary neoplasms					
12-Month interim evaluation	1				1
2-Year study	129	121	131	138	114
Total animals with benign neoplasms					
12-Month interim evaluation	1				1
2-Year study	50	50	48	50	48
Total benign neoplasms					
12-Month interim evaluation	1				1
2-Year study	94	105	114	112	99
Total animals with malignant neoplasms					
2-Year study	30	13	15	18	14
Total malignant neoplasms					
2-Year study	35	16	17	23	15
Total animals with metastatic neoplasms					
2-Year study	1	6		1	3
Total metastatic neoplasms					
2-Year study	1	10		1	4
Total animals with malignant neoplasms of uncertain primary site					
2-Year study		1			
Total animals with uncertain neoplasms-benign or malignant					
2-Year study				1	
Total uncertain neoplasms					
2-Year study				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 A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 0 ppm

Number of Days on Study	485	499	537	540	555	562	565	565	593	617	620	631	642	645	648	648	665	669	674	677	681	687	688	690	705
Carcass ID Number	001	035	042	017	030	034	029	056	002	054	008	028	055	022	043	051	014	027	004	018	006	049	009	047	044
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery						+				+			+	+	+		+				+		+		
Oral mucosa									+																
Squamous cell papilloma									X																
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																					X		X		
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X												
Carcinoma						X																			
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X	X					X		X	X	X				X	X						X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																						X			
Follicular cell, adenoma																			X						
Follicular cell, carcinoma						X																			
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X																		
Carcinoma																X									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma											X	X								X		X			
Interstitial cell, adenoma, multiple	X	X			X	X	X	X		X				X	X	X	X	X	X		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 Anthraquinone: 0 ppm

Number of Days on Study	708	724	724	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	024	012	053	007	013	016	025	038	039	048	005	011	019	020	021	023	026	032	041	045	050	052	057	059	060	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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
Hepatocellular adenoma					X																					1
Mesentery		+											+													10
Oral mucosa																										1
Squamous cell papilloma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma							X																			1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign								X					X							X	X					6
Bilateral, pheochromocytoma benign					X																		X			2
Islets, pancreatic	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X					X		X		X						X							X			15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma									X																	2
Follicular cell, adenoma																										1
Follicular cell, carcinoma																										1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X																X						3
Carcinoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma		X								X																6
Interstitial cell, adenoma, multiple	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	40

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

Number of Days on Study	485	499	537	540	555	562	565	565	593	617	620	631	642	645	648	648	665	669	674	677	681	687	688	690	705
Carcass ID Number	001	035	042	017	030	034	029	056	002	054	008	028	055	022	043	051	014	027	044	018	006	049	009	047	044
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+					+	+														+		+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	M	+	M	+	+	+	M	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Squamous cell papilloma						X																			
Trichoepithelioma																									
Sebaceous gland, adenoma																				X					
Subcutaneous tissue, fibroma																				X					
Subcutaneous tissue, schwannoma, malignant																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma	X																								
Tibia, chondroma															X										
Skeletal muscle	+																								
Osteosarcoma, metastatic, bone	X																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+																								
Spinal cord	+																								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																	X								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory epithelium, adenoma			X																						
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+																+								
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		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 Anthraquinone: 0 ppm

Number of Days on Study	708	724	724	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	024	012	053	007	013	016	025	038	039	048	005	011	019	020	021	023	026	032	041	045	050	052	057	059	060	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+			+			+																		8
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma									X																	1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Fibroadenoma																				X						1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma			X																							1
Squamous cell papilloma																										1
Trichoepithelioma																			X							1
Sebaceous gland, adenoma																										1
Subcutaneous tissue, fibroma			X												X	X				X						5
Subcutaneous tissue, schwannoma, malignant								X																		1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Tibia, chondroma																										1
Skeletal muscle																										1
Osteosarcoma, metastatic, bone																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma										X																2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory epithelium, adenoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																								X		1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X			X	X	X	X		X		X	X					X			X		X	X	X	25
Mesothelioma malignant					X								X													4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 469 ppm

Number of Days on Study	477	481	597	597	615	641	642	645	663	663	663	668	669	674	676	676	676	682	705	705	708	710	718	724	724
Carcass ID Number	067	055	077	092	088	087	085	095	076	081	097	064	061	016	080	083	093	074	082	102	062	072	084	075	099
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, salivary glands				X																					
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery									+																
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma				X																					
Schwannoma malignant								X																	
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									X
Pheochromocytoma benign							X								X		X			X					
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X			X	X	X			X	X		X	X		X		X		X					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, salivary glands				X																					
Schwannoma malignant, metastatic, salivary glands								X																	
C-cell, adenoma														X	X		X								
C-cell, carcinoma																									
Follicular cell, carcinoma	X																						X		
General Body System																									
None																									
Genital System																									
Coagulating gland																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X										X	X									X	
Carcinoma																									
Bilateral, adenoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 469 ppm

Number of Days on Study	724	724	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	100	107	063	066	068	070	073	078	090	091	094	098	109	065	069	071	079	086	089	096	101	103	104	108	110	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, salivary glands																										1
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
Hepatocellular adenoma						X									X					X						3
Mesentery					+		+											+								4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma																										1
Schwannoma malignant																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign						X				X	X															7
Bilateral, pheochromocytoma benign												X														1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, metastatic, thyroid gland															X										X	2
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X			X			X										X								15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, salivary glands																										1
Schwannoma malignant, metastatic, salivary glands																										1
C-cell, adenoma						X															X				X	6
C-cell, carcinoma															X										X	2
Follicular cell, carcinoma		X																								3
General Body System																										
None																										
Genital System																										
Coagulating gland																		+								1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																X	X					X		X		8
Carcinoma												X														1
Bilateral, adenoma					X																					1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 469 ppm

Number of Days on Study	477	481	597	597	615	641	642	645	663	663	663	668	669	674	676	677	676	682	705	705	708	710	718	724	724
Carcass ID Number	067	005	077	092	088	087	085	095	076	081	097	064	061	016	080	083	093	074	082	102	062	072	084	075	099
Genital System (continued)																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma							X				X		X		X										
Interstitial cell, adenoma, multiple	X		X	X		X		X	X	X		X		X			X		X	X	X	X	X	X	X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node												+							+						
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, salivary glands				X																					
Schwannoma malignant, metastatic, salivary glands								X																	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	M	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma	X																								X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma									X																
Subcutaneous tissue, fibroma												X											X		
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Chordoma, metastatic, uncertain primary site																					X				
Pheochromocytoma malignant, metastatic, adrenal medulla																									X
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, salivary glands				X																					
Special Senses System																									
None																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma													X												X
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear												X							X						
Mesothelioma malignant								X	X			X				X									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 469 ppm

Number of Days on Study	724	724	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	100	107	063	066	068	070	073	078	090	091	094	098	109	065	069	071	079	086	089	096	101	103	104	108	110	Total Tissues/ Tumors
Genital System (continued)																										
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma							X																			5
Interstitial cell, adenoma, multiple	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	41
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																		+								3
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, salivary glands																										1
Schwannoma malignant, metastatic, salivary glands																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																									X	1
Thymus	+	+	+	+	+	M	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	44
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Fibroadenoma					X					X									X							5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																				X						1
Keratoacanthoma															X											2
Subcutaneous tissue, fibroma													X											X		4
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X											X									2
Chordoma, metastatic, uncertain primary site																										1
Pheochromocytoma malignant, metastatic, adrenal medulla																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, salivary glands																										1
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																									X	3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma																	X									1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										2
Mesothelioma malignant																										4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 938 ppm

Number of Days on Study	312	477	561	565	596	603	603	620	638	643	648	648	663	666	680	682	687	699	704	705	705	705	719	719	723
Carcass ID Number	111	150	113	159	134	127	149	116	123	120	136	155	142	131	145	133	141	112	115	126	139	160	137	156	140
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery						+							+				+			+				+	
Oral mucosa																								+	
Squamous cell papilloma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+
Schwannoma malignant			X																						
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign										X					X					X			X	X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma								X				X				X					X				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+
C-cell, adenoma			X						X				X				X							X	
Follicular cell, adenoma																			X					X	
General Body System																									
Tissue NOS														+											
Chemodectoma benign														X											
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma										X									X	X		X			
Carcinoma							X																		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma				X	X							X													
Interstitial cell, adenoma, multiple			X			X	X		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 938 ppm

Number of Days on Study	723	724	724	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	153	118	132	117	119	124	125	128	130	135	143	144	151	154	158	114	121	122	129	138	146	147	148	152	157	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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
Hepatocellular adenoma							X		X					X								X				4
Mesentery									+																	6
Oral mucosa									+																	2
Squamous cell papilloma									X																	1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Schwannoma malignant																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign										X					X		X				X			X		10
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X															X							2
Carcinoma					X																					1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma		X		X	X										X					X			X	X		11
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma										X																6
Follicular cell, adenoma				X						X																4
General Body System																										
Tissue NOS																										1
Chemodectoma benign																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X								X											X			7
Carcinoma			X																							2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										3
Interstitial cell, adenoma, multiple	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	44

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 938 ppm

Number of Days on Study	312	477	561	565	596	603	603	620	638	643	648	648	663	666	680	682	687	699	704	705	705	705	719	719	723
Carcass ID Number	11	10	13	59	34	27	49	16	23	20	36	55	42	31	45	33	41	12	15	26	39	60	37	56	40
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node						+																			
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																								X	
Hemangiosarcoma																									
Thymus	+	+	+	+	+	M	+	+	+	M	M	+	+	+	+	+	M	+	M	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									X
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibrosarcoma																					X				
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant	X																								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye													+												
Zymbal's gland		+								+															
Carcinoma		X								X															
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, renal tubule, adenoma																									
Renal tubule, adenoma											X				X			X		X	X				
Renal tubule, oncocytoma benign																					X				
Transitional epithelium, papilloma									X																X
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																					X				
Mesothelioma malignant						X							X				X			X				X	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 938 ppm

Number of Days on Study	723	724	724	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	153	118	132	117	129	124	125	138	130	145	143	154	151	154	118	124	121	122	139	148	146	147	158	152	157	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma																										1
Hemangiosarcoma																								X		1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Fibroadenoma, multiple																		X								1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma													X						X							3
Subcutaneous tissue, fibroma																				X						1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, lipoma							X																			1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																		X								2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye			+		+																				+	4
Zymbal's gland																										2
Carcinoma																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, renal tubule, adenoma																	X									1
Renal tubule, adenoma	X																							X	X	8
Renal tubule, oncocytoma benign																										1
Transitional epithelium, papilloma																										2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma																				X				X	X	3
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Mesothelioma malignant																										5

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 1,875 ppm

Number of Days on Study	249	537	583	593	605	620	628	628	637	653	663	674	681	690	690	690	690	705	705	717	719	719	719	727	729
Carcass ID Number	172	187	162	182	185	204	167	170	171	179	199	194	186	174	180	197	203	163	198	161	166	188	192	164	165
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular adenoma																									
Hepatocellular adenoma, multiple																									
Histiocytic sarcoma																		X							
Leukemia megakaryocytic			X																						
Mesentery				+				+															+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																						X			
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma complex																									
Pheochromocytoma benign																								X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X	X											X		X			X		X	X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, c-cell, adenoma																									
C-cell, adenoma															X									X	
Follicular cell, adenoma																									
Follicular cell, carcinoma																	X								
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X				X														
Carcinoma															X						X				
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma													X			X								X	
Interstitial cell, adenoma, multiple			X	X	X	X	X	X	X	X	X	X		X	X		X	X	X	X	X	X	X		X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 1,875 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	168	176	177	181	183	184	189	190	191	195	201	205	208	210	169	173	175	178	193	196	200	202	206	207	209	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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
Hepatocellular carcinoma														X												1
Hepatocellular adenoma						X		X									X									3
Hepatocellular adenoma, multiple																		X								1
Histiocytic sarcoma																										1
Leukemia megakaryocytic																										1
Mesentery				+								+	+								+					7
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma complex												X														1
Pheochromocytoma benign			X		X						X											X			X	6
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																							X			1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma									X		X					X							X			11
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, c-cell, adenoma																			X							1
C-cell, adenoma	X								X	X		X														6
Follicular cell, adenoma															X											1
Follicular cell, carcinoma																										1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma	X									X																4
Carcinoma		X													X			X								5
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										3
Interstitial cell, adenoma, multiple	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	44

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 1,875 ppm

Number of Days on Study	249	537	583	593	605	620	628	628	637	653	663	674	681	690	690	690	690	705	705	717	719	719	719	727	729
Carcass ID Number	172	187	162	182	185	204	167	170	171	179	199	194	186	174	180	197	203	163	198	161	166	188	192	164	165
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																		+							
Mediastinal, histiocytic sarcoma																		X							
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma												X													
Histiocytic sarcoma																		X							
Leukemia megakaryocytic			X																						
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma										X	X		X	X						X					
Fibroadenoma, multiple																					X				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																							X		
Subcutaneous tissue, fibroma	X														X				X						
Subcutaneous tissue, histiocytic sarcoma																		X							
Subcutaneous tissue, melanoma malignant				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maxilla, osteosarcoma																						X			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																		X							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																						X			
Histiocytic sarcoma																		X							
Leukemia megakaryocytic			X																						
Osteosarcoma, metastatic, bone																						X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory epithelium, papilloma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye												+												+	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma														X						X					
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma		X		X					X													X			
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																		X							
Leukemia mononuclear												X											X		
Mesothelioma malignant				X				X															X		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 1,875 ppm

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	168	176	177	181	183	184	189	190	191	195	201	205	208	210	169	173	175	178	193	196	200	202	206	207	209	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										1
Mediastinal, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroma																										1
Histiocytic sarcoma																										1
Leukemia megakaryocytic																										1
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma											X				X											7
Fibroadenoma, multiple																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																				X						2
Subcutaneous tissue, fibroma						X					X															5
Subcutaneous tissue, histiocytic sarcoma																										1
Subcutaneous tissue, melanoma malignant																		X							X	3
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Maxilla, osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma													X													2
Histiocytic sarcoma																										1
Leukemia megakaryocytic																										1
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory epithelium, papilloma																X										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye					+	+									+											5
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma															X					X			X			5
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma									X					X								X				7
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear								X		X			X													5
Mesothelioma malignant													X													4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 3,750 ppm

Number of Days on Study	061	421	466	484	537	593	603	607	607	624	638	641	641	646	667	671	677	680	682	684	690	690	690	690	697
Carcass ID Number	236	222	245	225	218	268	213	216	253	214	234	230	270	244	241	231	243	223	219	232	226	242	265	267	261
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma									X																
Hepatocellular adenoma																									
Mesentery							+									+								+	+
Fibrosarcoma, metastatic, skin																									
Oral mucosa						+																			
Squamous cell carcinoma						X																			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth					+																				
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign												X				X					X				X
Bilateral, pheochromocytoma benign																								X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M	+	+	+	+	+
Adenoma																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X								X										
Thyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M	+	+	+	+	+
Bilateral, c-cell, adenoma																									
C-cell, adenoma																									
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma									X															X	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma		X	X						X																X
Interstitial cell, adenoma, multiple				X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 3,750 ppm

Number of Days on Study	699	705	724	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	
Carcass ID Number	237	262	229	211	220	227	235	240	249	251	256	257	258	260	264	266	212	215	233	238	239	248	254	255	259	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																										1
Hepatocellular adenoma				X						X																2
Mesentery	+																								+	6
Fibrosarcoma, metastatic, skin	X																									1
Oral mucosa																										1
Squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin	X																									1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma											X															1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign							X								X	X	X	X			X					10
Bilateral, pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Adenoma			X																							1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X											X			X		X			X			X	X	9
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Bilateral, c-cell, adenoma																									X	1
C-cell, adenoma							X			X			X													3
Follicular cell, adenoma													X													1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X											X							2
Carcinoma							X																			3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																										4
Interstitial cell, adenoma, multiple		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	44

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 3,750 ppm

Number of Days on Study	061	421	466	484	537	593	603	607	607	624	638	641	641	646	667	671	677	680	682	684	690	690	690	690	697
Carcass ID Number	236	222	245	225	218	268	213	216	253	214	234	230	270	244	241	231	243	223	219	232	226	242	265	267	261
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node								+																	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M
Fibroadenoma																	X								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Squamous cell papilloma																						X			
Subcutaneous tissue, fibroma																		X		X					
Subcutaneous tissue, fibrosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Meningioma malignant			X																						
Oligodendroglioma malignant																X									
Peripheral nerve				+									+												
Spinal cord				+									+												
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, preputial gland																								X	
Hepatocellular carcinoma, metastatic, liver									X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Special Senses System																									
Eye				+										+											
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma													X										X		
Transitional epithelium, papilloma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Transitional epithelium, papilloma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X			X		X							X					

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Anthraquinone: 3,750 ppm

Number of Days on Study	699	705	724	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	
Carcass ID Number	237	262	229	211	220	227	235	240	249	251	256	257	258	260	264	266	212	215	233	238	239	248	254	255	259	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																		+								2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	M	+	41
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibroadenoma						X											X		X							4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma			X	X								X						X					X			5
Squamous cell papilloma					X																					2
Subcutaneous tissue, fibroma																										2
Subcutaneous tissue, fibrosarcoma	X																									1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Meningioma malignant																										1
Oligodendroglioma malignant																										1
Peripheral nerve																										2
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, preputial gland																										1
Hepatocellular carcinoma, metastatic, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma										X																3
Transitional epithelium, papilloma												X														1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Transitional epithelium, papilloma						X									X								X			3
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																	X	X						X		7

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Adrenal Medulla: Benign Pheochromocytoma					
Overall rate[a]	8/50 (16%)	8/50 (16%)	10/50 (20%)	6/50 (12%)	11/50 (22%)
Adjusted rate[b]	19.5%	18.0%	23.1%	13.7%	26.5%
Terminal rate[c]	6/22 (27%)	4/23 (17%)	5/22 (23%)	5/26 (19%)	6/22 (27%)
First incidence (days)	681	642	643	727	641
Poly-3 test[d]	P=0.276	P=0.539N	P=0.446	P=0.336N	P=0.310
Adrenal Medulla: Benign, Malignant, or Complex Pheochromocytoma					
Overall rate	8/50 (16%)	9/50 (18%)	10/50 (20%)	7/50 (14%)	11/50 (22%)
Adjusted rate	19.5%	20.2%	23.1%	16.0%	26.5%
Terminal rate	6/22 (27%)	4/23 (17%)	5/22 (23%)	6/26 (23%)	6/22 (27%)
First incidence (days)	681	642	643	727	641
Poly-3-test	P=0.301	P=0.574	P=0.446	P=0.446N	P=0.310
Kidney (Renal Tubule): Adenoma					
Overall rate	1/50 (2%)	3/50 (6%)	9/50 (18%)	5/50 (10%)	3/50 (6%)
Adjusted rate	2.5%	6.8%	20.7%	11.4%	7.3%
Terminal rate	1/22 (5%)	1/23 (4%)	3/22 (14%)	3/26 (12%)	1/22 (5%)
First incidence (days)	729 (T)	669	648	690	641
Poly-3 test	P=0.474	P=0.333	P=0.010	P=0.119	P=0.308
Liver: Hepatocellular Adenoma					
Overall rate	1/50 (2%)	3/50 (6%)	4/50 (8%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.5%	6.9%	9.4%	9.1%	4.9%
Terminal rate	1/22 (5%)	3/23 (13%)	4/22 (18%)	4/26 (15%)	2/22 (9%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.518	P=0.330	P=0.192	P=0.200	P=0.499
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate	1/50 (2%)	3/50 (6%)	4/50 (8%)	5/50 (10%)	3/50 (6%)
Adjusted rate	2.5%	6.9%	9.4%	11.4%	7.3%
Terminal rate	1/22 (5%)	3/23 (13%)	4/22 (18%)	5/26 (19%)	2/22 (9%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)	607
Poly-3 test	P=0.310	P=0.330	P=0.192	P=0.118	P=0.307
Mammary Gland: Fibroadenoma					
Overall rate	1/50 (2%)	5/50 (10%)	1/50 (2%)	8/50 (16%)	4/50 (8%)
Adjusted rate	2.5%	11.3%	2.3%	17.9%	9.8%
Terminal rate	1/22 (5%)	3/23 (13%)	1/22 (5%)	2/26 (8%)	3/22 (14%)
First incidence (days)	729 (T)	477	729 (T)	653	677
Poly-3 test	P=0.143	P=0.122	P=0.750N	P=0.022	P=0.178
Pancreatic Islets: Adenoma or Carcinoma					
Overall rate	2/49 (4%)	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	4.9%	0.0%	7.0%	0.0%	0.0%
Terminal rate	0/21 (0%)	0/23 (0%)	3/22 (14%)	0/26 (0%)	0/22 (0%)
First incidence (days)	562	—[e]	729 (T)	—	—
Poly-3 test	P=0.139N	P=0.221N	P=0.523	P=0.220N	P=0.236N

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	15/50 (30%)	15/50 (30%)	11/49 (22%)	11/50 (22%)	9/50 (18%)
Adjusted rate	34.0%	32.0%	25.6%	24.3%	21.8%
Terminal rate	5/22 (23%)	3/23 (13%)	6/22 (27%)	4/26 (15%)	6/22 (27%)
First incidence (days)	537	481	620	537	603
Poly-3 test	P=0.100N	P=0.509N	P=0.266N	P=0.216N	P=0.151N
Preputial Gland: Adenoma					
Overall rate	3/50 (6%)	9/49 (18%)	7/50 (14%)	4/49 (8%)	2/50 (4%)
Adjusted rate	7.3%	20.5%	16.2%	9.2%	4.9%
Terminal rate	2/22 (9%)	5/23 (22%)	3/22 (14%)	2/25 (8%)	2/22 (9%)
First incidence (days)	565	597	643	628	729 (T)
Poly-3 test	P=0.092N	P=0.072	P=0.175	P=0.527	P=0.507N
Preputial Gland: Carcinoma					
Overall rate	1/50 (2%)	1/49 (2%)	2/50 (4%)	5/49 (10%)	3/50 (6%)
Adjusted rate	2.4%	2.3%	4.6%	11.7%	7.3%
Terminal rate	0/22 (0%)	1/23 (4%)	0/22 (0%)	3/25 (12%)	1/22 (5%)
First incidence (days)	648	729 (T)	603	690	607
Poly-3 test	P=0.114	P=0.750N	P=0.518	P=0.111	P=0.306
Preputial Gland: Adenoma or Carcinoma					
Overall rate	4/50 (8%)	10/49 (20%)	9/50 (18%)	9/49 (18%)	5/50 (10%)
Adjusted rate	9.6%	22.8%	20.6%	20.7%	12.1%
Terminal rate	2/22 (9%)	6/23 (26%)	3/22 (14%)	5/25 (20%)	3/22 (14%)
First incidence (days)	565	597	603	628	607
Poly-3 test	P=0.399N	P=0.086	P=0.133	P=0.131	P=0.495
Skin: Keratoacanthoma					
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	2/50 (4%)	5/50 (10%)
Adjusted rate	0.0%	4.6%	7.0%	4.6%	12.3%
Terminal rate	0/22 (0%)	1/23 (4%)	2/22 (9%)	1/26 (4%)	4/22 (18%)
First incidence (days)	—	663	723	719	724
Poly-3 test	P=0.027	P=0.254	P=0.127	P=0.253	P=0.029
Skin: Squamous Cell Papilloma or Keratoacanthoma					
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	2/50 (4%)	7/50 (14%)
Adjusted rate	2.4%	4.6%	7.0%	4.6%	17.2%
Terminal rate	0/22 (0%)	1/23 (4%)	2/22 (9%)	1/26 (4%)	5/22 (23%)
First incidence (days)	562	663	723	719	690
Poly-3 test	P=0.008	P=0.522	P=0.317	P=0.521	P=0.027
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, or Basal Cell Adenoma					
Overall rate	3/50 (6%)	3/50 (6%)	3/50 (6%)	2/50 (4%)	7/50 (14%)
Adjusted rate	7.3%	6.8%	7.0%	4.6%	17.2%
Terminal rate	1/22 (5%)	2/23 (9%)	2/22 (9%)	1/26 (4%)	5/22 (23%)
First incidence (days)	562	663	723	719	690
Poly-3 test	P=0.064	P=0.634N	P=0.647N	P=0.473N	P=0.149

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Skin (Subcutaneous Tissue): Fibroma					
Overall rate	5/50 (10%)	4/50 (8%)	1/50 (2%)	5/50 (10%)	2/50 (4%)
Adjusted rate	12.2%	9.1%	2.3%	11.1%	4.9%
Terminal rate	3/22 (14%)	2/23 (9%)	1/22 (5%)	2/26 (8%)	0/22 (0%)
First incidence (days)	677	668	729 (T)	249	680
Poly-3 test	P=0.272N	P=0.455N	P=0.091N	P=0.571N	P=0.214N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma					
Overall rate	5/50 (10%)	4/50 (8%)	2/50 (4%)	5/50 (10%)	3/50 (6%)
Adjusted rate	12.2%	9.1%	4.7%	11.1%	7.3%
Terminal rate	3/22 (14%)	2/23 (9%)	1/22 (5%)	2/26 (8%)	0/22 (0%)
First incidence (days)	677	668	705	249	680
Poly-3 test	P=0.407N	P=0.455N	P=0.197N	P=0.571N	P=0.353N
Skin (Subcutaneous Tissue): Malignant Melanoma					
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	0.0%	6.8%	0.0%
Terminal rate	0/22 (0%)	0/23 (0%)	0/22 (0%)	2/26 (8%)	0/22 (0%)
First incidence (days)	—	—	—	593	—
Poly-3 test	P=0.387	—[f]	—	P=0.134	—
Testes: Adenoma					
Overall rate	46/50 (92%)	46/50 (92%)	47/50 (94%)	47/50 (94%)	48/50 (96%)
Adjusted rate	95.8%	94.8%	98.0%	97.0%	98.2%
Terminal rate	22/22 (100%)	23/23 (100%)	22/22 (100%)	25/26 (96%)	22/22 (100%)
First incidence (days)	485	477	561	583	421
Poly-3 test	P=0.248	P=0.627N	P=0.483	P=0.605	P=0.450
Thyroid Gland (Follicular Cell): Adenoma					
Overall rate	1/50 (2%)	0/50 (0%)	4/48 (8%)	1/50 (2%)	1/47 (2%)
Adjusted rate	2.4%	0.0%	9.7%	2.3%	2.6%
Terminal rate	0/22 (0%)	0/23 (0%)	2/22 (9%)	1/26 (4%)	1/22 (5%)
First incidence (days)	674	—	704	729 (T)	729 (T)
Poly-3 test	P=0.597N	P=0.487N	P=0.179	P=0.746N	P=0.747
Thyroid Gland (Follicular Cell): Carcinoma					
Overall rate	1/50 (2%)	3/50 (6%)	0/48 (0%)	1/50 (2%)	0/47 (0%)
Adjusted rate	2.4%	6.8%	0.0%	2.3%	0.0%
Terminal rate	0/22 (0%)	0/23 (0%)	0/22 (0%)	0/26 (0%)	0/22 (0%)
First incidence (days)	562	477	—	690	—
Poly-3 test	P=0.168N	P=0.332	P=0.501N	P=0.747N	P=0.515N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma					
Overall rate	2/50 (4%)	3/50 (6%)	4/48 (8%)	2/50 (4%)	1/47 (2%)
Adjusted rate	4.8%	6.8%	9.7%	4.6%	2.6%
Terminal rate	0/22 (0%)	0/23 (0%)	2/22 (9%)	1/26 (4%)	1/22 (5%)
First incidence (days)	562	477	704	690	729 (T)
Poly-3 test	P=0.267N	P=0.532	P=0.333	P=0.674N	P=0.527N

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Thyroid Gland (C-cell): Adenoma					
Overall rate	2/50 (4%)	6/50 (12%)	6/48 (13%)	7/50 (14%)	4/47 (9%)
Adjusted rate	4.9%	13.6%	14.2%	16.0%	10.4%
Terminal rate	1/22 (5%)	3/23 (13%)	1/22 (5%)	5/26 (19%)	4/22 (18%)
First incidence (days)	687	674	561	690	729 (T)
Poly-3 test	P=0.403	P=0.159	P=0.143	P=0.095	P=0.307
Thyroid Gland (C-cell): Adenoma or Carcinoma					
Overall rate	2/50 (4%)	7/50 (14%)	6/48 (13%)	7/50 (14%)	4/47 (9%)
Adjusted rate	4.9%	15.8%	14.2%	16.0%	10.4%
Terminal rate	1/22 (5%)	4/23 (17%)	1/22 (5%)	5/26 (19%)	4/22 (18%)
First incidence (days)	687	674	561	690	729 (T)
Poly-3 test	P=0.465	P=0.098	P=0.143	P=0.095	P=0.307
Urinary Bladder: Papilloma					
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	7/50 (14%)	3/49 (6%)
Adjusted rate	0.0%	2.3%	7.0%	15.5%	7.6%
Terminal rate	0/22 (0%)	1/23 (4%)	3/22 (14%)	3/26 (12%)	3/22 (14%)
First incidence (days)	—	729 (T)	729 (T)	537	729 (T)
Poly-3 test	P=0.053	P=0.514	P=0.127	P=0.011	P=0.113
All Organs: Malignant Mesothelioma					
Overall rate	4/50 (8%)	4/50 (8%)	5/50 (10%)	4/50 (8%)	0/50 (0%)
Adjusted rate	9.7%	9.0%	11.5%	9.0%	0.0%
Terminal rate	2/22 (9%)	0/23 (0%)	0/22 (0%)	1/26 (4%)	0/22 (0%)
First incidence (days)	617	645	603	593	—
Poly-3 test	P=0.053N	P=0.601N	P=0.533	P=0.602N	P=0.062N
All Organs: Mononuclear Cell Leukemia					
Overall rate	25/50 (50%)	2/50 (4%)	1/50 (2%)	5/50 (10%)	7/50 (14%)
Adjusted rate	56.4%	4.5%	2.3%	11.4%	16.7%
Terminal rate	12/22 (55%)	0/23 (0%)	0/22 (0%)	3/26 (12%)	3/22 (14%)
First incidence (days)	499	668	705	674	607
Poly-3 test	P=0.003N	P<0.001N	P<0.001N	P<0.001N	P<0.001N
All Organs: Benign Neoplasms					
Overall rate	50/50 (100%)	50/50 (100%)	48/50 (96%)	50/50 (100%)	48/50 (96%)
Adjusted rate	100.0%	100.0%	99.3%	100.0%	98.2%
Terminal rate	22/22 (100%)	23/23 (100%)	22/22 (100%)	26/26 (100%)	22/22 (100%)
First incidence (days)	485	477	561	249	421
Poly-3 test	P=0.212N	—	P=0.985N	—	P=0.549N
All Organs: Malignant Neoplasms					
Overall rate	30/50 (60%)	14/50 (28%)	15/50 (30%)	18/50 (36%)	14/50 (28%)
Adjusted rate	64.8%	30.4%	32.1%	39.8%	31.9%
Terminal rate	13/22 (59%)	3/23 (13%)	3/22 (14%)	9/26 (35%)	4/22 (18%)
First incidence (days)	485	477	312	593	466
Poly-3 test	P=0.026N	P<0.001N	P<0.001N	P=0.011N	P<0.001N

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
All Organs: Benign or Malignant Neoplasms					
Overall rate	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%	100.0%
Terminal rate	22/22 (100%)	23/23 (100%)	22/22 (100%)	26/26 (100%)	22/22 (100%)
First incidence (days)	485	477	312	249	421
Poly-3 test	P=1.000N	—	—	—	P=1.000N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, kidney, liver, pancreatic islets, pituitary gland, preputial gland, testis, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidences 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 Renal Tubule Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratory			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	0/50	0/50	0/50
Manganese (II) sulfate monohydrate	1/52	0/52	1/52
Oxazepam	1/50	0/50	1/50
Pentachlorophenol	1/50	0/50	1/50
Primadone	2/50	0/50	2/50
Triamterene	1/50	0/50	1/50
Tricresyl phosphate	0/51	0/51	0/51
Overall Historical Incidence			
Total (%)	7/902 (0.8%)	0/902	7/902 (0.8%)
Mean ± standard deviation	0.8% ± 1.2%		0.8% ± 1.2%
Range	0%-4%		0%-4%

[a] Data as of November 10, 1998

TABLE A4b
Historical Incidence of Kidney Transitional Epithelial Papillomas in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Battelle Columbus Laboratory	
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	0/50
Manganese (II) sulfate monohydrate	0/52
Oxazepam	0/50
Pentachlorophenol	0/50
Primadone	0/50
Triamterene	0/50
Tricresyl phosphate	0/51
Overall Historical Incidence	
Total (%)	1/902 (0.1%)
Mean ± standard deviation	0.1% ± 0.5%
Range	0%-2%

[a] Data as of November 10, 1998

TABLE A4c
Historical Incidence of Urinary Bladder Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Papilloma	Carcinoma	Papilloma or Carcinoma
Historical Incidence at Battelle Columbus Laboratory			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	0/49	0/49	0/49
Manganese (II) sulfate monohydrate	1/52	0/52	1/52
Oxazepam	0/50	0/50	0/50
Pentachlorophenol	1/48	0/48	1/48
Primadone	0/50	0/50	0/50
Triamterene	0/49	0/49	0/49
Tricresyl phosphate	0/51	0/51	0/51
Overall Historical Incidence			
Total (%)	2/891 (0.2%)	1/891 (0.1%)	3/891 (0.3%)
Mean ± standard deviation	0.2% ± 0.7%	0.1% ± 0.5%	0.3% ± 0.8%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of November 10, 1998

TABLE A4d
Historical Incidence of Hepatocellular Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratory			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	1/50	0/50	1/50
Manganese (II) sulfate monohydrate	0/52	0/52	0/52
Oxazepam	1/50	1/50	2/50
Pentachlorophenol	0/50	0/50	0/50
Primadone	1/50	0/50	1/50
Triamterene	0/50	0/50	0/50
Tricresyl phosphate	0/50	0/50	0/50
Overall Historical Incidence			
Total (%)	21/902 (2.3%)	7/902 (0.8%)	26/902 (2.9%)
Mean ± standard deviation	2.3% ± 3.2%	0.8% ± 1.6%	2.9% ± 3.5%
Range	0%-10%	0%-6%	0%-10%

[a] Data as of November 10, 1998

TABLE A4e
Historical Incidence of Mononuclear Cell Leukemia in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Battelle Columbus Laboratory	
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	30/50
Manganese (II) sulfate monohydrate	32/52
Oxazepam	27/50
Pentachlorophenol	25/50
Primadone	35/50
Triamterene	22/50
Tricresyl phosphate	20/51
Overall Historical Incidence	
Total (%)	494/904 (54.7%)
Mean ± standard deviation	54.7% ± 11.2%
Range	32%-74%

[a] Data as of November 10, 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 Anthraquinone[a]

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Disposition Summary					
Animals initially in study	60	50	50	50	60
3-Month interim evaluation	5				5
12-Month interim evaluation	5				5
Early deaths					
Moribund	21	21	16	14	17
Natural deaths	7	6	12	10	11
Survivors					
Died last week of study	1				
Terminal sacrifice	21	23	22	26	22
Animals examined microscopically	60	50	50	50	60
3-Month Interim Evaluation					
Alimentary System					
Liver	(5)				(5)
Centrilobular, hypertrophy					5 (100%)
Pancreas	(5)				(5)
Atrophy					1 (20%)
Cardiovascular System					
Heart	(5)				(5)
Cardiomyopathy	3 (60%)				3 (60%)
Endocrine System					
Thyroid gland	(5)				(5)
Follicular cell, hypertrophy					5 (100%)
Hematopoietic System					
Spleen	(5)				(5)
Accessory spleen	1 (20%)				
Congestion					5 (100%)
Capsule, hyperplasia					1 (20%)
Respiratory System					
Lung	(5)				(5)
Inflammation					3 (60%)
Nose	(5)				(5)
Inflammation					1 (20%)
Urinary System					
Kidney	(5)				(5)
Accumulation, hyaline droplet					5 (100%)
Nephropathy	5 (100%)				5 (100%)

[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 Anthraquinone

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
3-Month Interim Evaluation (continued)					
Systems Examined with No Lesions Observed					
General Body System					
Genital System					
Integumentary System					
Musculoskeletal System					
Nervous System					
Special Senses System					
12-Month Interim Evaluation					
Alimentary System					
Intestine large, rectum	(5)				(5)
Parasite metazoan	2 (40%)				4 (80%)
Intestine large, cecum	(5)				(5)
Ulcer					1 (20%)
Liver	(5)				(5)
Basophilic focus	2 (40%)				
Hepatodiaphragmatic nodule					1 (20%)
Inflammation	4 (80%)				3 (60%)
Necrosis	4 (80%)				1 (20%)
Vacuolization cytoplasmic	4 (80%)				5 (100%)
Bile duct, hyperplasia	4 (80%)				4 (80%)
Centrilobular, hypertrophy					5 (100%)
Cardiovascular System					
Heart	(5)				(5)
Cardiomyopathy	5 (100%)				5 (100%)
Endocrine System					
Pituitary gland	(5)				(5)
Pars distalis, hyperplasia	1 (20%)				1 (20%)
Genital System					
Epididymis	(5)				(5)
Inflammation	2 (40%)				
Preputial gland	(5)				(5)
Inflammation	5 (100%)				3 (60%)
Prostate	(5)				(5)
Inflammation	3 (60%)				4 (80%)
Testes	(5)				(5)
Atrophy	1 (20%)				
Interstitial cell, hyperplasia	4 (80%)				4 (80%)
Hematopoietic System					
Bone marrow	(5)				(5)
Hyperplasia					3 (60%)
Thymus	(5)				(5)
Atrophy	2 (40%)				4 (80%)

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
12-Month Interim Evaluation (continued)					
Respiratory System					
Lung	(5)				(5)
Inflammation	1 (20%)				2 (40%)
Nose	(5)				(5)
Inflammation	1 (20%)				1 (20%)
Urinary System					
Kidney	(5)				(5)
Accumulation, hyaline droplet					5 (100%)
Nephropathy	5 (100%)				5 (100%)
Medulla, mineralization					5 (100%)
Urinary bladder	(5)				(5)
Inflammation					1 (20%)
Systems Examined with No Lesions Observed					
General Body System					
Integumentary System					
Musculoskeletal System					
Nervous System					
Special Senses System					
2-Year Study					
Alimentary System					
Esophagus	(50)	(50)	(50)	(50)	(49)
Inflammation		1 (2%)			
Ulcer		1 (2%)	1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)	(50)
Edema				1 (2%)	
Inflammation				1 (2%)	
Mineralization				1 (2%)	
Parasite metazoan	1 (2%)	1 (2%)	1 (2%)		
Intestine large, rectum	(50)	(50)	(50)	(50)	(50)
Mineralization				1 (2%)	
Parasite metazoan	5 (10%)	7 (14%)	7 (14%)	5 (10%)	3 (6%)
Intestine large, cecum	(50)	(50)	(50)	(50)	(49)
Edema	1 (2%)				
Inflammation	2 (4%)				2 (4%)
Mineralization					1 (2%)
Artery, inflammation				1 (2%)	
Intestine small, duodenum	(50)	(50)	(50)	(50)	(50)
Erosion		1 (2%)	1 (2%)		1 (2%)
Inflammation					2 (4%)
Ulcer	2 (4%)	1 (2%)	1 (2%)		
Intestine small, jejunum	(50)	(50)	(50)	(50)	(50)
Inflammation					1 (2%)
Ulcer		1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(50)	(50)
Inflammation		1 (2%)	1 (2%)	1 (2%)	

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Alimentary System (continued)					
Liver	(50)	(50)	(50)	(50)	(50)
Angiectasis	6 (12%)	21 (42%)	13 (26%)	9 (18%)	9 (18%)
Basophilic focus	25 (50%)	35 (70%)	35 (70%)	32 (64%)	23 (46%)
Clear cell focus	9 (18%)	5 (10%)	6 (12%)	8 (16%)	5 (10%)
Congestion	1 (2%)				
Degeneration, cystic	9 (18%)	31 (62%)	36 (72%)	28 (56%)	29 (58%)
Eosinophilic focus	9 (18%)	22 (44%)	30 (60%)	29 (58%)	20 (40%)
Hematopoietic cell proliferation	2 (4%)	1 (2%)	3 (6%)	3 (6%)	
Hemorrhage				1 (2%)	
Hepatodiaphragmatic nodule	5 (10%)	9 (18%)	2 (4%)	5 (10%)	4 (8%)
Inflammation	13 (26%)	30 (60%)	28 (56%)	30 (60%)	27 (54%)
Mineralization				1 (2%)	
Mixed cell focus	4 (8%)	12 (24%)	15 (30%)	13 (26%)	10 (20%)
Necrosis	5 (10%)	5 (10%)	5 (10%)	7 (14%)	5 (10%)
Pigmentation	1 (2%)				
Thrombosis	1 (2%)	1 (2%)			1 (2%)
Vacuolization cytoplasmic	5 (10%)	18 (36%)	23 (46%)	17 (34%)	23 (46%)
Bile duct, cyst	1 (2%)		1 (2%)		
Bile duct, hyperplasia	48 (96%)	50 (100%)	47 (94%)	49 (98%)	47 (94%)
Centrilobular, degeneration	2 (4%)	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Centrilobular, hypertrophy		4 (8%)	21 (42%)	13 (26%)	29 (58%)
Centrilobular, necrosis		1 (2%)		2 (4%)	1 (2%)
Mesentery	(10)	(4)	(6)	(7)	(6)
Inflammation	2 (20%)				
Artery, inflammation				1 (14%)	
Artery, mineralization					2 (33%)
Fat, inflammation				1 (14%)	
Fat, necrosis	6 (60%)	3 (75%)	2 (33%)	2 (29%)	4 (67%)
Oral mucosa	(1)		(2)		(1)
Pharyngeal, hyperplasia			1 (50%)		
Pancreas	(50)	(50)	(50)	(50)	(50)
Atrophy	26 (52%)	30 (60%)	25 (50%)	28 (56%)	24 (48%)
Hyperplasia	2 (4%)	4 (8%)	2 (4%)	2 (4%)	1 (2%)
Hypertrophy, focal	1 (2%)	6 (12%)	3 (6%)	2 (4%)	2 (4%)
Inflammation		2 (4%)	2 (4%)	1 (2%)	
Artery, hypertrophy					1 (2%)
Artery, inflammation	1 (2%)	1 (2%)	3 (6%)	1 (2%)	5 (10%)
Artery, mineralization				1 (2%)	3 (6%)
Duct, cyst					1 (2%)
Duct, hyperplasia	1 (2%)				
Salivary glands	(50)	(50)	(48)	(50)	(47)
Atrophy	1 (2%)	3 (6%)			1 (2%)
Hyperplasia			1 (2%)		
Inflammation		1 (2%)			
Mineralization				1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)	(50)
Edema	2 (4%)	4 (8%)	2 (4%)	2 (4%)	3 (6%)
Foreign body	1 (2%)				
Hyperplasia	4 (8%)	11 (22%)	7 (14%)	4 (8%)	4 (8%)
Inflammation	4 (8%)		2 (4%)		1 (2%)
Mineralization				2 (4%)	4 (8%)
Perforation		1 (2%)	1 (2%)		
Ulcer	3 (6%)	8 (16%)	4 (8%)		3 (6%)

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Alimentary System (continued)					
Stomach, glandular	(50)	(50)	(50)	(50)	(50)
Erosion	2 (4%)	4 (8%)	1 (2%)	2 (4%)	1 (2%)
Fibrosis	1 (2%)	1 (2%)			
Hyperplasia					1 (2%)
Inflammation	1 (2%)		1 (2%)	1 (2%)	1 (2%)
Mineralization	2 (4%)	4 (8%)	7 (14%)	10 (20%)	10 (20%)
Ulcer	3 (6%)	2 (4%)	3 (6%)	1 (2%)	3 (6%)
Tooth					(1)
Inflammation					1 (100%)
Cardiovascular System					
Blood vessel	(50)	(50)	(50)	(50)	(50)
Mineralization	3 (6%)	3 (6%)	4 (8%)	7 (14%)	7 (14%)
Aorta, aneurysm					1 (2%)
Heart	(50)	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)		
Cardiomyopathy	42 (84%)	44 (88%)	44 (88%)	43 (86%)	41 (82%)
Inflammation		1 (2%)	1 (2%)	2 (4%)	1 (2%)
Mineralization	1 (2%)	2 (4%)	4 (8%)	5 (10%)	5 (10%)
Artery, inflammation		1 (2%)			1 (2%)
Atrium, thrombosis	6 (12%)		1 (2%)	1 (2%)	
Endocrine System					
Adrenal cortex	(50)	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule			1 (2%)		
Degeneration, cystic	7 (14%)	4 (8%)	3 (6%)	7 (14%)	9 (18%)
Hematopoietic cell proliferation		1 (2%)			
Hemorrhage			1 (2%)	2 (4%)	
Hyperplasia	8 (16%)	10 (20%)	8 (16%)	13 (26%)	6 (12%)
Hypertrophy	4 (8%)	1 (2%)	2 (4%)	4 (8%)	1 (2%)
Necrosis					1 (2%)
Pigmentation	1 (2%)				
Thrombosis			1 (2%)		1 (2%)
Vacuolization cytoplasmic	3 (6%)	2 (4%)		1 (2%)	4 (8%)
Adrenal medulla	(50)	(50)	(50)	(50)	(50)
Angiectasis					1 (2%)
Hyperplasia	17 (34%)	24 (48%)	22 (44%)	20 (40%)	15 (30%)
Thrombosis					1 (2%)
Parathyroid gland	(49)	(48)	(48)	(50)	(45)
Hyperplasia	5 (10%)	13 (27%)	19 (40%)	20 (40%)	12 (27%)
Hyperplasia, focal	1 (2%)	1 (2%)		1 (2%)	1 (2%)
Inflammation					1 (2%)
Pituitary gland	(50)	(50)	(49)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)		1 (2%)	1 (2%)
Cyst	2 (4%)	3 (6%)	3 (6%)	3 (6%)	6 (12%)
Hemorrhage	1 (2%)		1 (2%)		
Thrombosis				1 (2%)	
Pars distalis, hyperplasia	13 (26%)	26 (52%)	24 (49%)	28 (56%)	19 (38%)
Pars intermedia, hyperplasia		1 (2%)		2 (4%)	1 (2%)
Pars nervosa, hyperplasia	5 (10%)	1 (2%)		4 (8%)	1 (2%)
Thyroid gland	(50)	(50)	(48)	(50)	(47)
C-cell, hyperplasia	14 (28%)	22 (44%)	14 (29%)	13 (26%)	12 (26%)
Follicle, cyst	3 (6%)	2 (4%)	3 (6%)	5 (10%)	4 (9%)
Follicular cell, hyperplasia	3 (6%)	3 (6%)	1 (2%)	1 (2%)	3 (6%)
Follicular cell, hypertrophy		1 (2%)			

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
General Body System					
None					
Genital System					
Coagulating gland		(1)			
Inflammation		1 (100%)			
Epididymis	(50)	(50)	(50)	(50)	(50)
Granuloma sperm		1 (2%)		3 (6%)	1 (2%)
Inflammation				1 (2%)	1 (2%)
Mineralization		1 (2%)		1 (2%)	1 (2%)
Preputial gland	(50)	(49)	(50)	(49)	(50)
Atrophy					1 (2%)
Hyperplasia	3 (6%)	4 (8%)	3 (6%)		1 (2%)
Infiltration cellular			1 (2%)		
Inflammation	42 (84%)	34 (69%)	43 (86%)	37 (76%)	43 (86%)
Duct, ectasia	1 (2%)	4 (8%)	4 (8%)	4 (8%)	
Prostate	(50)	(50)	(50)	(50)	(50)
Atrophy				1 (2%)	
Cyst		1 (2%)			1 (2%)
Hyperplasia	1 (2%)			1 (2%)	1 (2%)
Inflammation	33 (66%)	37 (74%)	36 (72%)	31 (62%)	34 (68%)
Metaplasia, squamous			1 (2%)		
Mineralization				1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(49)	(50)
Dilatation			2 (4%)		
Hyperplasia		1 (2%)			
Inflammation	1 (2%)	1 (2%)		1 (2%)	1 (2%)
Mineralization	1 (2%)		1 (2%)	2 (4%)	3 (6%)
Testes	(50)	(50)	(50)	(50)	(50)
Atrophy	6 (12%)	10 (20%)	7 (14%)	6 (12%)	5 (10%)
Thrombosis				1 (2%)	
Artery, inflammation	1 (2%)			1 (2%)	
Interstitial cell, hyperplasia	27 (54%)	27 (54%)	26 (52%)	24 (48%)	26 (52%)
Hematopoietic System					
Bone marrow	(50)	(50)	(50)	(50)	(50)
Hemorrhage	2 (4%)	2 (4%)		1 (2%)	1 (2%)
Hyperplasia	25 (50%)	28 (56%)	37 (74%)	36 (72%)	33 (66%)
Myelofibrosis	1 (2%)			1 (2%)	
Necrosis	1 (2%)				
Lymph node	(8)	(3)	(1)	(1)	(2)
Mediastinal, ectasia			1 (100%)		
Mediastinal, hematopoietic cell proliferation	1 (13%)				
Mediastinal, pigmentation	1 (13%)				
Renal, hyperplasia, plasma cell		1 (33%)			
Lymph node, mandibular	(50)	(50)	(47)	(50)	(47)
Atrophy				1 (2%)	1 (2%)
Ectasia	8 (16%)	2 (4%)	2 (4%)	1 (2%)	4 (9%)
Hyperplasia, plasma cell	3 (6%)	2 (4%)		1 (2%)	3 (6%)
Inflammation	1 (2%)	1 (2%)			
Necrosis	1 (2%)				
Lymph node, mesenteric	(50)	(49)	(50)	(50)	(49)
Angiectasis				1 (2%)	
Atrophy	2 (4%)		1 (2%)	1 (2%)	2 (4%)
Ectasia	3 (6%)	5 (10%)	7 (14%)	7 (14%)	7 (14%)

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Hematopoietic System (continued)					
Lymph node, mesenteric (continued)	(50)	(49)	(50)	(50)	(49)
Hemorrhage	2 (4%)	1 (2%)			
Necrosis	1 (2%)				
Spleen	(50)	(50)	(50)	(50)	(50)
Accessory spleen	1 (2%)		1 (2%)		1 (2%)
Angiectasis			1 (2%)		
Congestion	6 (12%)	35 (70%)	37 (74%)	30 (60%)	31 (62%)
Fibrosis	5 (10%)		3 (6%)	2 (4%)	2 (4%)
Hematopoietic cell proliferation	37 (74%)	45 (90%)	44 (88%)	43 (86%)	39 (78%)
Hemorrhage	2 (4%)				
Infarct	1 (2%)				
Infiltration cellular		2 (4%)		1 (2%)	1 (2%)
Necrosis	1 (2%)				
Pigmentation	12 (24%)	36 (72%)	38 (76%)	33 (66%)	28 (56%)
Capsule, fibrosis	1 (2%)				
Capsule, hyperplasia					1 (2%)
Lymphoid follicle, atrophy	1 (2%)		2 (4%)	2 (4%)	6 (12%)
Red pulp, depletion cellular	3 (6%)	3 (6%)	2 (4%)	4 (8%)	2 (4%)
Thymus	(46)	(44)	(45)	(46)	(41)
Atrophy	40 (87%)	43 (98%)	44 (98%)	46 (100%)	41 (100%)
Necrosis	1 (2%)				
Epithelial cell, hyperplasia		1 (2%)			
Integumentary System					
Mammary gland	(45)	(47)	(46)	(50)	(47)
Cyst		1 (2%)			
Hyperplasia	10 (22%)	21 (45%)	12 (26%)	12 (24%)	10 (21%)
Mineralization		1 (2%)			
Duct, hyperplasia		1 (2%)			
Skin	(50)	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)		1 (2%)	1 (2%)	
Hemorrhage				1 (2%)	
Hyperplasia				1 (2%)	
Inflammation					2 (4%)
Dermis, fibrosis			1 (2%)	1 (2%)	
Hair follicle, atrophy		1 (2%)			
Subcutaneous tissue, inflammation, granulomatous		1 (2%)			
Musculoskeletal System					
Bone	(50)	(50)	(50)	(50)	(50)
Fibrous osteodystrophy	2 (4%)	4 (8%)	8 (16%)	11 (22%)	9 (18%)
Fracture					1 (2%)
Necrosis	1 (2%)				
Osteomalacia		13 (26%)	12 (24%)	8 (16%)	3 (6%)
Osteopetrosis	1 (2%)				
Nervous System					
Brain	(50)	(50)	(50)	(50)	(50)
Hemorrhage	2 (4%)				1 (2%)
Inflammation, suppurative			1 (2%)		
Cerebrum, degeneration	1 (2%)				

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Respiratory System					
Lung	(50)	(50)	(50)	(50)	(50)
Cyst, squamous					1 (2%)
Foreign body		1 (2%)	2 (4%)	1 (2%)	
Hemorrhage			1 (2%)	2 (4%)	2 (4%)
Inflammation	11 (22%)	13 (26%)	13 (26%)	8 (16%)	14 (28%)
Mineralization	1 (2%)	2 (4%)	3 (6%)	7 (14%)	6 (12%)
Necrosis				1 (2%)	
Pigmentation	3 (6%)	6 (12%)	4 (8%)	5 (10%)	5 (10%)
Thrombosis	1 (2%)			2 (4%)	
Alveolar epithelium, hyperplasia	3 (6%)	9 (18%)	9 (18%)	7 (14%)	3 (6%)
Mediastinum, inflammation		1 (2%)			
Mediastinum, thrombosis				1 (2%)	
Nose	(50)	(50)	(50)	(50)	(50)
Foreign body	4 (8%)	3 (6%)	7 (14%)	5 (10%)	6 (12%)
Hemorrhage		1 (2%)			
Inflammation	4 (8%)	3 (6%)	8 (16%)	6 (12%)	6 (12%)
Respiratory epithelium, inflammation	1 (2%)				3 (6%)
Trachea	(50)	(50)	(50)	(50)	(49)
Inflammation			1 (2%)		1 (2%)
Special Senses System					
Eye	(2)		(4)	(5)	(2)
Cataract	2 (100%)		2 (50%)	4 (80%)	
Degeneration				1 (20%)	
Hemorrhage				2 (40%)	
Inflammation			1 (25%)		1 (50%)
Mineralization				4 (80%)	1 (50%)
Cornea, inflammation			1 (25%)	1 (20%)	
Retina, degeneration	2 (100%)		3 (75%)	4 (80%)	1 (50%)
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	3 (6%)	14 (28%)	10 (20%)	16 (32%)	16 (32%)
Cyst	3 (6%)	4 (8%)	9 (18%)	7 (14%)	13 (26%)
Hydronephrosis	2 (4%)		2 (4%)		2 (4%)
Infarct	1 (2%)				1 (2%)
Inflammation	7 (14%)	5 (10%)	2 (4%)	5 (10%)	4 (8%)
Mineralization, diffuse	3 (6%)	2 (4%)	9 (18%)	8 (16%)	6 (12%)
Nephropathy	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)
Pigmentation	25 (50%)	31 (62%)	36 (72%)	38 (76%)	33 (66%)
Thrombosis				1 (2%)	
Medulla, mineralization	30 (60%)	42 (84%)	46 (92%)	47 (94%)	49 (98%)
Renal tubule, hyperplasia	3 (6%)	7 (14%)	3 (6%)	9 (18%)	9 (18%)
Renal tubule, hyperplasia, oncocytic				1 (2%)	1 (2%)
Transitional epithelium, hyperplasia	28 (56%)	45 (90%)	44 (88%)	48 (96%)	48 (96%)
Urinary bladder	(50)	(50)	(50)	(50)	(49)
Hemorrhage			2 (4%)		1 (2%)
Inflammation	8 (16%)	8 (16%)	11 (22%)	8 (16%)	9 (18%)
Serosa, inflammation			1 (2%)		
Transitional epithelium, hyperplasia		1 (2%)	1 (2%)	1 (2%)	

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

TABLE B1 Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Anthraquinone B-2
TABLE B2 Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone B-6
TABLE B3 Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Anthraquinone B-26
TABLE B4a Historical Incidence of Renal Tubule Neoplasms in Untreated Female F344/N Rats B-29
TABLE B4b Historical Incidence of Urinary Bladder Neoplasms in Untreated Female F344/N Rats B-29
TABLE B4c Historical Incidence of Hepatocellular Neoplasms in Untreated Female F344/N Rats B-30
TABLE B4d Historical Incidence of Mononuclear Cell Leukemia in Untreated Female F344/N Rats B-30
TABLE B5 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Anthraquinone B-31

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Disposition Summary					
Animals initially in study	60	50	50	50	60
3-Month interim evaluation	5				5
12-Month interim evaluation	5				5
Early deaths					
Moribund	14	7	12	7	6
Natural deaths	13	3	3	6	4
Survivors					
Terminal sacrifice	23	40	35	37	40
Animals examined microscopically	60	50	50	50	60
Systems Examined at 3 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					
Esophagus	(50)	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(49)	(50)	(50)
Leiomyosarcoma					1 (2%)
Intestine large, cecum	(50)	(49)	(49)	(50)	(49)
Intestine small, jejunum	(50)	(50)	(50)	(50)	(49)
Leiomyosarcoma	1 (2%)				
Liver	(50)	(50)	(50)	(50)	(49)
Carcinoma, metastatic, islets, pancreatic			1 (2%)		
Fibrous histiocytoma, metastatic, tissue NOS	1 (2%)				
Hepatocellular carcinoma	1 (2%)				
Hepatocellular adenoma		2 (4%)	5 (10%)	3 (6%)	3 (6%)
Hepatocellular adenoma, multiple			1 (2%)	1 (2%)	
Mesentery	(4)	(4)	(6)	(3)	(2)
Carcinoma, metastatic, islets, pancreatic			1 (17%)		
Carcinoma, metastatic, kidney				1 (33%)	
Oral mucosa	(1)		(1)		
Pharyngeal, squamous cell papilloma			1 (100%)		
Pancreas	(50)	(50)	(50)	(50)	(49)
Carcinoma, metastatic, islets, pancreatic			1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)	(48)
Stomach, glandular	(50)	(50)	(50)	(50)	(49)
Tongue					(1)
Squamous cell papilloma					1 (100%)

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Cardiovascular System					
Heart	(50)	(50)	(50)	(50)	(48)
Carcinoma, metastatic, mammary gland					1 (2%)
Schwannoma benign					1 (2%)
Endocrine System					
Adrenal cortex	(50)	(50)	(50)	(50)	(49)
Adenoma		1 (2%)			
Carcinoma, metastatic, kidney				1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)	(49)
Pheochromocytoma malignant			1 (2%)		
Pheochromocytoma benign			1 (2%)	1 (2%)	2 (4%)
Islets, pancreatic	(49)	(50)	(50)	(50)	(49)
Carcinoma		1 (2%)	1 (2%)		
Parathyroid gland	(42)	(44)	(48)	(48)	(41)
Pituitary gland	(50)	(50)	(50)	(49)	(49)
Squamous cell carcinoma, metastatic, nose			1 (2%)		
Pars distalis, adenoma	20 (40%)	22 (44%)	23 (46%)	17 (35%)	19 (39%)
Thyroid gland	(50)	(50)	(50)	(50)	(49)
C-cell, adenoma	5 (10%)	4 (8%)	5 (10%)	10 (20%)	9 (18%)
C-cell, carcinoma					1 (2%)
Follicular cell, adenoma	1 (2%)				1 (2%)
Follicular cell, carcinoma			1 (2%)		
General Body System					
Tissue NOS	(1)				
Fibrous histiocytoma	1 (100%)				
Genital System					
Clitoral gland	(49)	(46)	(48)	(49)	(48)
Adenoma	2 (4%)	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Carcinoma	2 (4%)	1 (2%)	2 (4%)		
Bilateral, adenoma	1 (2%)	1 (2%)	1 (2%)		
Ovary	(50)	(50)	(50)	(50)	(49)
Carcinoma, metastatic, kidney				1 (2%)	
Granulosa cell tumor malignant			1 (2%)		
Granulosa-theca tumor malignant					1 (2%)
Granulosa-theca tumor benign		1 (2%)			
Luteoma	1 (2%)				
Oviduct		(1)	(1)		
Carcinoma, metastatic, islets, pancreatic			1 (100%)		
Uterus	(50)	(50)	(50)	(50)	(49)
Polyp stromal	6 (12%)	9 (18%)	9 (18%)	4 (8%)	5 (10%)
Sarcoma stromal		1 (2%)			
Schwannoma malignant					1 (2%)
Cervix, leiomyosarcoma				2 (4%)	
Cervix, polyp stromal	1 (2%)				

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Hematopoietic System					
Bone marrow	(50)	(50)	(50)	(50)	(50)
Lymph node	(10)		(1)	(1)	
Lumbar, fibrous histiocytoma, metastatic, tissue NOS	1 (10%)				
Mediastinal, carcinoma, metastatic, islets, pancreatic			1 (100%)		
Mediastinal, carcinoma, metastatic, kidney				1 (100%)	
Lymph node, mandibular	(49)	(49)	(50)	(48)	(47)
Lymph node, mesenteric	(50)	(49)	(49)	(50)	(49)
Carcinoma, metastatic, kidney				1 (2%)	
Spleen	(50)	(50)	(50)	(50)	(49)
Hemangiosarcoma	1 (2%)	1 (2%)		1 (2%)	
Thymus	(46)	(48)	(48)	(49)	(46)
Integumentary System					
Mammary gland	(50)	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			1 (2%)	
Adenoma, multiple	1 (2%)				
Carcinoma	1 (2%)		1 (2%)	2 (4%)	3 (6%)
Fibroadenoma	19 (38%)	20 (40%)	20 (40%)	18 (36%)	13 (26%)
Fibroadenoma, multiple	6 (12%)	6 (12%)	6 (12%)	6 (12%)	6 (12%)
Skin	(50)	(50)	(50)	(50)	(50)
Keratoacanthoma		1 (2%)			
Trichoepithelioma					1 (2%)
Subcutaneous tissue, fibroma			1 (2%)		
Musculoskeletal System					
Bone	(50)	(50)	(50)	(50)	(50)
Mandible, carcinosarcoma, metastatic, Zymbal's gland	1 (2%)				
Nervous System					
Brain	(50)	(50)	(50)	(50)	(49)
Astrocytoma malignant			1 (2%)	1 (2%)	
Oligodendroglioma malignant			1 (2%)		
Respiratory System					
Lung	(50)	(50)	(50)	(50)	(48)
Alveolar/bronchiolar adenoma		1 (2%)	2 (4%)		
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)			1 (2%)
Carcinoma, metastatic, islets, pancreatic			1 (2%)		
Carcinoma, metastatic, kidney				1 (2%)	
Carcinoma, metastatic, mammary gland					1 (2%)
Carcinosarcoma, metastatic, Zymbal's gland	1 (2%)				
Squamous cell carcinoma, metastatic, nose			1 (2%)		
Nose	(50)	(50)	(50)	(50)	(50)
Nasolacrimal duct, squamous cell carcinoma			1 (2%)		
Trachea	(50)	(50)	(50)	(50)	(49)

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Special Senses System					
Harderian gland			(1)		
Squamous cell carcinoma, metastatic, nose			1 (100%)		
Zymbal's gland	(1)	(1)			
Carcinoma		1 (100%)			
Carcinosarcoma	1 (100%)				
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(49)
Carcinoma, metastatic, islets, pancreatic			1 (2%)		
Fibrous histiocytoma, metastatic, tissue NOS	1 (2%)				
Bilateral, renal tubule, adenoma			1 (2%)	1 (2%)	
Renal tubule, adenoma		4 (8%)	7 (14%)	6 (12%)	12 (24%)
Renal tubule, adenoma, multiple			1 (2%)		
Renal tubule, carcinoma		2 (4%)		1 (2%)	2 (4%)
Renal tubule, carcinoma, metastatic, kidney					1 (2%)
Urinary bladder	(49)	(49)	(49)	(50)	(49)
Transitional epithelium, carcinoma					1 (2%)
Transitional epithelium, papilloma				1 (2%)	1 (2%)
Systemic Lesions					
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)
Leukemia mononuclear	18 (36%)	1 (2%)	1 (2%)	2 (4%)	
Neoplasm Summary					
Total animals with primary neoplasms[c]					
2-Year study	46	46	47	42	45
Total primary neoplasms					
2-Year study	91	83	98	80	86
Total animals with benign neoplasms					
2-Year study	39	45	44	40	41
Total benign neoplasms					
2-Year study	64	74	87	71	75
Total animals with malignant neoplasms					
2-Year study	24	9	11	9	10
Total malignant neoplasms					
2-Year study	27	9	11	9	11
Total animals with metastatic neoplasms					
2-Year study	2		2	1	2
Total metastatic neoplasms					
2-Year study	5		10	6	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 Anthraquinone: 0 ppm

Number of Days on Study	550	571	571	575	605	617	617	635	645	645	649	652	662	665	666	676	676	681	689	702	703	704	713	715	718
Carcass ID Number	272	290	325	328	309	285	308	289	286	296	311	278	294	305	301	275	314	291	327	276	300	283	282	315	277
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, tissue NOS																								X	
Hepatocellular carcinoma		X																							
Mesentery			+											+							+				
Oral mucosa													+												
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X								X				X		X			X	X		X	X			X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma	X																								
Follicular cell, adenoma																									
General Body System																									
Tissue NOS																								+	
Fibrous histiocytoma																								X	
Genital System																									
Clitoral gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																			X						
Carcinoma																				X					
Bilateral, adenoma																						X			
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Luteoma																			X						
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal					X					X	X	X													
Cervix, polyp 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 Anthraquinone: 0 ppm

Number of Days on Study	718	718	730	730	730	730	730	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	280	302	271	293	304	318	321	273	281	295	312	313	316	320	324	274	279	292	297	303	306	307	323	326	330	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																							X			1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, tissue NOS																										1
Hepatocellular carcinoma																										1
Mesentery																					+					4
Oral mucosa																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	M	M	42
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X		X	X	X		X		X					X					X	X		X		X		20
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma			X															X	X	X						5
Follicular cell, adenoma																								X		1
General Body System																										
Tissue NOS																										1
Fibrous histiocytoma																										1
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																			X							2
Carcinoma	X																									2
Bilateral, adenoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Luteoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal																			X		X					6
Cervix, polyp stromal																										1
Vagina																										1

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

Number of Days on Study	550	571	571	575	605	617	617	635	645	645	649	652	662	665	666	676	676	681	689	702	703	704	713	715	718
Carcass ID Number	272	290	325	328	309	285	308	289	286	296	311	278	294	305	301	275	314	291	327	276	300	283	282	315	277
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+	+		+				+		+		+		+					+	+	
Lumbar, fibrous histiocytoma, metastatic, tissue NOS																								X	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adenoma, multiple																									
Carcinoma		X																							
Fibroadenoma			X		X		X			X			X	X	X	X	X		X				X	X	X
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mandible, carcinosarcoma, metastatic, Zymbal's gland																	X								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma					X																				
Carcinosarcoma, metastatic, Zymbal's gland																	X								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																	+								
Zymbal's gland																	+								
Carcinosarcoma																	X								
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, tissue NOS																								X	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X	X	X	X	X			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 Anthraquinone: 0 ppm

Number of Days on Study	718	718	730	730	730	730	730	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	280	302	271	293	304	318	321	273	281	295	312	313	316	320	324	274	279	292	297	303	306	307	323	326	330	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+																								10
Lumbar, fibrous histiocytoma, metastatic, tissue NOS																										1
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma									X																	1
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma						X																				1
Adenoma, multiple																								X		1
Carcinoma																										1
Fibroadenoma			X		X						X		X	X											X	19
Fibroadenoma, multiple	X					X				X							X	X						X		6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mandible, carcinosarcoma, metastatic, Zymbal's gland																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																										1
Carcinosarcoma, metastatic, Zymbal's gland																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																					+					2
Zymbal's gland																										1
Carcinosarcoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, tissue NOS																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X			X															X						18

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 469 ppm

Number of Days on Study	513	575	589	592	606	645	647	679	694	718	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731
Carcass ID Number	361	311	352	445	359	370	362	350	372	376	332	334	335	338	341	343	347	354	356	358	360	365	366	379	333
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery	+												+											+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X		
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X	X	X		X			X	X			X	X	X		X									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																							X		X
General Body System																									
None																									
Genital System																									
Clitoral gland	+	M	+	+	+	+	M	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X			X		
Carcinoma						X																			
Bilateral, adenoma																									X
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa-theca tumor benign																									
Oviduct																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal				X							X							X		X					
Sarcoma stromal																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 469 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	337	339	340	344	349	351	355	363	367	368	377	380	336	342	346	348	353	357	364	369	371	373	374	375	378	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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma										X										X						2
Mesentery								+																		4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma											X															1
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	M	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X				X	X		X	X	X	X		X	X					X			X	X			22
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma					X	X																				4
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoma																										2
Carcinoma																										1
Bilateral, adenoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa-theca tumor benign																					X					1
Oviduct																				+						1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal		X					X			X							X				X					9
Sarcoma stromal																					X					1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 469 ppm

Number of Days on Study	513	575	589	592	606	645	647	679	694	718	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731
Carcass ID Number	361	331	352	445	459	570	662	550	572	776	732	734	735	738	741	743	747	754	756	758	760	765	766	779	733
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma		X					X			X				X		X				X	X			X	X
Fibroadenoma, multiple				X	X										X										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X																					
Alveolar/bronchiolar carcinoma								X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																						+			
Zymbal's gland			+																						
Carcinoma			X																						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Renal tubule, carcinoma																								X	
Urinary bladder	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																			X						

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 469 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	337	339	340	344	349	351	355	363	367	368	377	380	336	342	346	348	353	357	364	369	371	373	374	375	378	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma					X																					1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma	X			X				X	X	X	X	X		X		X		X					X			20
Fibroadenoma, multiple						X							X											X		6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																						X				1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Alveolar/bronchiolar carcinoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye											+								+							3
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma			X						X		X						X									4
Renal tubule, carcinoma																X										2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 938 ppm

Number of Days on Study	085	506	570	571	571	625	638	652	655	676	703	703	718	723	723	730	730	730	730	730	730	730	730	730	730
Carcass ID Number	412	428	413	392	407	420	382	415	391	405	389	419	394	410	427	381	390	395	397	398	402	408	411	414	423
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, islets, pancreatic													X												
Hepatocellular adenoma										X						X				X					
Hepatocellular adenoma, multiple																									
Mesentery												+	+				+								
Carcinoma, metastatic, islets, pancreatic													X												
Oral mucosa									+																
Pharyngeal, squamous cell papilloma									X																
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, islets, pancreatic													X												
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign																						X			
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma													X												
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, nose							X																		
Pars distalis, adenoma						X		X			X	X	X	X	X				X	X	X			X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma							X								X					X			X		
Follicular cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma														X											
Carcinoma				X						X															
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																									
Oviduct													+												
Carcinoma, metastatic, islets, pancreatic													X												
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal									X									X							

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 938 ppm

Number of Days on Study	730	730	730	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732		
Carcass ID Number	424	426	429	383	385	386	388	393	396	406	409	416	417	418	425	384	387	399	400	401	403	404	421	422	430	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, islets, pancreatic																										1
Hepatocellular adenoma				X				X																		5
Hepatocellular adenoma, multiple																		X								1
Mesentery					+															+					+	6
Carcinoma, metastatic, islets, pancreatic																										1
Oral mucosa																										1
Pharyngeal, squamous cell papilloma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, islets, pancreatic																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth							+																			1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																	X									1
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, nose																										1
Pars distalis, adenoma	X	X			X	X	X	X								X		X	X			X	X	X		23
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma							X																			5
Follicular cell, carcinoma						X																				1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma						X				X																3
Carcinoma																										2
Bilateral, adenoma																						X				1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor malignant												X														1
Oviduct																										1
Carcinoma, metastatic, islets, pancreatic																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal									X		X					X	X				X	X		X		9

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 938 ppm

Number of Days on Study	085	056	570	571	571	625	638	652	655	676	703	703	718	723	723	730	730	730	730	730	730	730	730	730	730
Carcass ID Number	412	428	413	392	407	420	382	415	391	405	389	419	394	410	427	381	390	395	397	398	402	408	411	414	423
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node													+												
Mediastinal, carcinoma, metastatic, islets, pancreatic													X												
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma					X	X		X	X			X			X					X	X				X
Fibroadenoma, multiple										X				X											
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma												X													
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant	X																								
Oligodendroglioma malignant		X																							
Peripheral nerve									+																
Spinal cord									+																
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																		X			X				
Carcinoma, metastatic, islets, pancreatic													X												
Squamous cell carcinoma, metastatic, nose							X																		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nasolacrimal duct, squamous cell Carcinoma							X																		
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye							+				+														
Harderian gland							+																		
Squamous cell carcinoma, metastatic, nose							X																		
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, islets, pancreatic													X												
Bilateral, renal tubule, adenoma																					X				
Renal tubule, adenoma			X																						X
Renal tubule, adenoma, multiple																						X			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 938 ppm

Number of Days on Study	730	730	730	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	424	426	429	383	385	386	388	393	396	406	409	416	417	418	425	384	387	399	400	401	403	404	421	422	430	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										1
Mediastinal, carcinoma, metastatic, islets, pancreatic																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma								X																		1
Fibroadenoma		X	X		X	X		X				X			X			X		X				X	X	20
Fibroadenoma, multiple	X									X						X			X							6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Oligodendroglioma malignant																										1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										2
Carcinoma, metastatic, islets, pancreatic																										1
Squamous cell carcinoma, metastatic, nose																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nasolacrimal duct, squamous cell Carcinoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										2
Harderian gland																										1
Squamous cell carcinoma, metastatic, nose																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, islets, pancreatic																										1
Bilateral, renal tubule, adenoma																										1
Renal tubule, adenoma	X										X				X		X						X			7
Renal tubule, adenoma, multiple																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X																1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 1,875 ppm

Number of Days on Study	550	611	634	635	638	647	675	689	701	704	722	723	723	730	730	730	730	730	730	730	730	730	730	731	731
Carcass ID Number	431	442	452	453	480	439	472	433	458	463	443	447	460	432	434	448	449	450	451	455	464	469	477	437	444
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma													X												
Hepatocellular adenoma, multiple																							X		
Mesentery		+																							
Carcinoma, metastatic, kidney		X																							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, kidney		X																							
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Pars distalis, adenoma	X				X		X		X	X		X	X		X				X			X			X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma								X		X				X								X		X	X
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, kidney		X																							
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal					X									X											
Cervix, leiomyosarcoma				X																					

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 1,875 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	445	446	456	457	461	465	466	467	470	471	473	474	435	436	438	440	441	454	459	462	468	475	476	478	479	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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
Hepatocellular adenoma	X			X																						3
Hepatocellular adenoma, multiple																										1
Mesentery										+															+	3
Carcinoma, metastatic, kidney																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, kidney																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign				X																						1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma								X				X		X			X		X		X					17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma			X			X			X						X											10
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma	X																									2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, kidney																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal															X			X								4
Cervix, leiomyosarcoma					X																					2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 1,875 ppm

Number of Days on Study	550	611	634	635	638	647	675	689	701	704	722	723	723	730	730	730	730	730	730	730	730	730	730	731	731
Carcass ID Number	431	442	452	453	480	439	472	433	458	463	443	447	460	432	434	448	449	450	451	455	464	469	477	437	444
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+																							
Mediastinal, carcinoma, metastatic, kidney		X																							
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, kidney		X																							
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma					X										X										
Fibroadenoma		X				X	X			X		X	X			X	X			X	X	X	X		X
Fibroadenoma, multiple								X			X													X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant						X																			
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, kidney		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye									+																+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, renal tubule, adenoma																									
Renal tubule, adenoma				X							X												X		
Renal tubule, carcinoma		X																							
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X																						

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 1,875 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	445	446	456	457	461	465	466	467	470	471	473	474	435	436	438	440	441	454	459	462	468	475	476	478	479	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										1
Mediastinal, carcinoma, metastatic, kidney																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, kidney																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																	X									1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																		X								1
Carcinoma																										2
Fibroadenoma												X			X				X	X					X	18
Fibroadenoma, multiple								X						X							X					6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, kidney																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																				+						3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, renal tubule, adenoma											X															1
Renal tubule, adenoma			X																		X	X				6
Renal tubule, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma																									X	1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear												X														2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 3,750 ppm

Number of Days on Study	486	536	689	699	709	712	718	718	722	724	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730
Carcass ID Number	523	506	501	483	498	509	526	530	539	512	481	487	488	489	492	495	497	503	504	508	510	518	521	522	524
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma					X																				
Intestine large, cecum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery																									
Pancreas	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Squamous cell papilloma																									
Cardiovascular System																									
Blood vessel	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, mammary gland						X																			
Schwannoma benign																									
Endocrine System																									
Adrenal cortex	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign		X	X																						
Islets, pancreatic	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	M	+	+	M	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X				X		X				X					X		X			X	X	
Thyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																			X				X	X	
C-cell, carcinoma																									X
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma		X																							
Ovary	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa-theca tumor malignant																									
Uterus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal			X					X																	
Schwannoma malignant												X													
Vagina																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 3,750 ppm

Number of Days on Study	730	730	731	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	529	536	484	499	502	505	507	515	516	517	519	520	531	540	485	486	493	494	501	525	528	532	533	535	537	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma			X							X		X														3
Mesentery	+			+																						2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue													+													1
Squamous cell papilloma													X													1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, metastatic, mammary gland																										1
Schwannoma benign					X																					1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	M	+	41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X							X		X	X	X		X	X	X		X					X	X		19
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma						X		X						X							X		X	X		9
C-cell, carcinoma																										1
Follicular cell, adenoma												X														1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Granulosa-theca tumor malignant							X																			1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp stromal		X		X																					X	5
Schwannoma malignant																										1
Vagina																							+			1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 3,750 ppm

Number of Days on Study	486	536	689	699	709	712	718	718	722	724	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730
Carcass ID Number	523	506	501	483	498	509	526	530	539	512	481	487	488	489	492	495	497	503	504	508	510	518	521	522	524
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma						X	X																		
Fibroadenoma		X		X	X		X	X										X	X						
Fibroadenoma, multiple											X					X									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trichoepithelioma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve					+																				
Spinal cord					+																				
Respiratory System																									
Lung	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma							X																		
Carcinoma, metastatic, mammary gland						X																			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye															+										
Urinary System																									
Kidney	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma			X		X	X										X	X				X				
Renal tubule, carcinoma														X						X					
Renal tubule, carcinoma, metastatic, kidney																				X					
Urinary bladder	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, carcinoma																									
Transitional epithelium, papilloma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Anthraquinone: 3,750 ppm

Number of Days on Study	730	730	731	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	529	536	484	499	502	505	507	515	516	517	519	520	531	540	485	486	493	494	501	525	528	532	533	535	537	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																							X			3
Fibroadenoma		X							X						X			X		X	X					13
Fibroadenoma, multiple										X	X	X		X												6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trichoepithelioma									X																	1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar carcinoma																										1
Carcinoma, metastatic, mammary gland																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye				+					+			+				+										5
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Renal tubule, adenoma					X			X			X					X		X	X							12
Renal tubule, carcinoma																										2
Renal tubule, carcinoma, metastatic, kidney																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Transitional epithelium, carcinoma			X																							1
Transitional epithelium, papilloma																						X				1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Clitoral Gland: Adenoma					
Overall rate[a]	3/49 (6%)	3/46 (7%)	4/48 (8%)	2/49 (4%)	1/48 (2%)
Adjusted rate[b]	7.0%	6.9%	9.3%	4.3%	2.1%
Terminal rate[c]	1/23 (4%)	3/39 (8%)	3/34 (9%)	2/37 (5%)	0/38 (0%)
First incidence (days)	689	730 (T)	723	730 (T)	536
Poly-3 test[d]	P=0.132N	P=0.655N	P=0.503	P=0.467N	P=0.275N
Clitoral Gland: Adenoma or Carcinoma					
Overall rate	5/49 (10%)	4/46 (9%)	6/48 (13%)	2/49 (4%)	1/48 (2%)
Adjusted rate	11.6%	9.1%	13.7%	4.3%	2.1%
Terminal rate	1/23 (4%)	3/39 (8%)	3/34 (9%)	2/37 (5%)	0/38 (0%)
First incidence (days)	689	645	571	730 (T)	536
Poly-3 test	P=0.032N	P=0.488N	P=0.513	P=0.188N	P=0.083N
Kidney (Renal Tubule): Adenoma					
Overall rate	0/50 (0%)	4/50 (8%)	9/50 (18%)	7/50 (14%)	12/49 (24%)
Adjusted rate	0.0%	8.6%	19.8%	14.8%	25.2%
Terminal rate	0/23 (0%)	4/40 (10%)	8/35 (23%)	5/37 (14%)	9/40 (23%)
First incidence (days)	—[e]	730 (T)	570	635	689
Poly-3 test	P<0.001	P=0.071	P=0.002	P=0.011	P<0.001
Kidney (Renal Tubule): Adenoma or Carcinoma					
Overall rate	0/50 (0%)	6/50 (12%)	9/50 (18%)	8/50 (16%)	14/49 (29%)
Adjusted rate	0.0%	12.9%	19.8%	16.7%	29.5%
Terminal rate	0/23 (0%)	6/40 (15%)	8/35 (23%)	5/37 (14%)	11/40 (28%)
First incidence (days)	—	730 (T)	570	611	689
Poly-3 test	P<0.001	P=0.020	P=0.002	P=0.006	P<0.001
Liver: Hepatocellular Adenoma					
Overall rate	0/50 (0%)	2/50 (4%)	6/50 (12%)	4/50 (8%)	3/49 (6%)
Adjusted rate	0.0%	4.3%	13.3%	8.5%	6.4%
Terminal rate	0/23 (0%)	2/40 (5%)	5/35 (14%)	3/37 (8%)	3/40 (8%)
First incidence (days)	—	730 (T)	676	723	730 (T)
Poly-3 test	P=0.298	P=0.255	P=0.018	P=0.072	P=0.136
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate	1/50 (2%)	2/50 (4%)	6/50 (12%)	4/50 (8%)	3/49 (6%)
Adjusted rate	2.3%	4.3%	13.3%	8.5%	6.4%
Terminal rate	0/23 (0%)	2/40 (5%)	5/35 (14%)	3/37 (8%)	3/40 (8%)
First incidence (days)	571	730 (T)	676	723	730 (T)
Poly-3 test	P=0.398	P=0.523	P=0.062	P=0.203	P=0.335
Mammary Gland: Fibroadenoma					
Overall rate	25/50 (50%)	26/50 (52%)	26/50 (52%)	24/50 (48%)	19/50 (38%)
Adjusted rate	54.1%	53.9%	55.6%	49.7%	38.8%
Terminal rate	11/23 (48%)	21/40 (53%)	18/35 (51%)	16/37 (43%)	14/40 (35%)
First incidence (days)	571	575	571	611	536
Poly-3 test	P=0.040N	P=0.575N	P=0.528	P=0.411N	P=0.095N

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Mammary Gland: Fibroadenoma or Adenoma					
Overall rate	25/50 (50%)	26/50 (52%)	26/50 (52%)	25/50 (50%)	19/50 (38%)
Adjusted rate	54.1%	53.9%	55.6%	51.8%	38.8%
Terminal rate	11/23 (48%)	21/40 (53%)	18/35 (51%)	17/37 (46%)	14/40 (35%)
First incidence (days)	571	575	571	611	536
Poly-3 test	P=0.044N	P=0.575N	P=0.528	P=0.491N	P=0.095N
Mammary Gland: Carcinoma					
Overall rate	1/50 (2%)	0/50 (0%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	2.3%	0.0%	2.2%	4.2%	6.2%
Terminal rate	0/23 (0%)	0/40 (0%)	1/35 (3%)	1/37 (3%)	1/40 (3%)
First incidence (days)	571	—	730 (T)	638	712
Poly-3 test	P=0.079	P=0.487N	P=0.753N	P=0.529	P=0.343
Mammary Gland: Adenoma or Carcinoma					
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	6.9%	0.0%	2.2%	6.3%	6.2%
Terminal rate	2/23 (9%)	0/40 (0%)	1/35 (3%)	2/37 (5%)	1/40 (3%)
First incidence (days)	571	—	730 (T)	638	712
Poly-3 test	P=0.272	P=0.107N	P=0.293N	P=0.623N	P=0.614N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma					
Overall rate	26/50 (52%)	26/50 (52%)	26/50 (52%)	27/50 (54%)	21/50 (42%)
Adjusted rate	55.6%	53.9%	55.6%	55.5%	42.8%
Terminal rate	11/23 (48%)	21/40 (53%)	18/35 (51%)	18/37 (49%)	15/40 (38%)
First incidence (days)	571	575	571	611	536
Poly-3 test	P=0.102N	P=0.515N	P=0.580N	P=0.578N	P=0.144N
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	20/50 (40%)	22/50 (44%)	23/50 (46%)	17/49 (35%)	19/49 (39%)
Adjusted rate	44.4%	45.0%	50.1%	35.9%	39.5%
Terminal rate	10/23 (44%)	16/40 (40%)	16/35 (46%)	10/36 (28%)	16/40 (40%)
First incidence (days)	550	513	625	550	689
Poly-3 test	P=0.220N	P=0.559	P=0.369	P=0.264N	P=0.395N
Thyroid Gland (C-cell): Adenoma					
Overall rate	5/50 (10%)	4/50 (8%)	5/50 (10%)	10/50 (20%)	9/49 (18%)
Adjusted rate	11.5%	8.6%	11.0%	21.2%	18.8%
Terminal rate	4/23 (17%)	4/40 (10%)	3/35 (9%)	8/37 (22%)	9/40 (23%)
First incidence (days)	550	730 (T)	638	689	730 (T)
Poly-3 test	P=0.068	P=0.460N	P=0.606N	P=0.168	P=0.248
Thyroid Gland (C-cell): Adenoma or Carcinoma					
Overall rate	5/50 (10%)	4/50 (8%)	5/50 (10%)	10/50 (20%)	10/49 (20%)
Adjusted rate	11.5%	8.6%	11.0%	21.2%	20.9%
Terminal rate	4/23 (17%)	4/40 (10%)	3/35 (9%)	8/37 (22%)	10/40 (25%)
First incidence (days)	550	730 (T)	638	689	730 (T)
Poly-3 test	P=0.036	P=0.460N	P=0.606N	P=0.168	P=0.175

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Uterus: Stromal Polyp					
Overall rate	7/50 (14%)	9/50 (18%)	9/50 (18%)	4/50 (8%)	5/50 (10%)
Adjusted rate	15.7%	19.2%	19.9%	8.4%	10.4%
Terminal rate	2/23 (9%)	8/40 (20%)	8/35 (23%)	3/37 (8%)	3/40 (8%)
First incidence (days)	605	592	655	638	689
Poly-3 test	P=0.104N	P=0.437	P=0.405	P=0.226N	P=0.323N
Uterus: Stromal Polyp or Stromal Sarcoma					
Overall rate	7/50 (14%)	9/50 (18%)	9/50 (18%)	4/50 (8%)	5/50 (10%)
Adjusted rate	15.7%	19.2%	19.9%	8.4%	10.4%
Terminal rate	2/23 (9%)	8/40 (20%)	8/35 (23%)	3/37 (8%)	3/40 (8%)
First incidence (days)	605	592	655	638	689
Poly-3 test	P=0.104N	P=0.437	P=0.405	P=0.226N	P=0.323N
All Organs: Mononuclear Cell Leukemia					
Overall rate	18/50 (36%)	1/50 (2%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate	38.0%	2.2%	2.2%	4.2%	0.0%
Terminal rate	2/23 (9%)	1/40 (3%)	1/35 (3%)	1/37 (3%)	0/40 (0%)
First incidence (days)	571	730 (T)	730 (T)	634	—
Poly-3 test	P<0.001N	P<0.001N	P<0.001N	P<0.001N	P<0.001N
All Organs: Benign Neoplasms					
Overall rate	39/50 (78%)	45/50 (90%)	44/50 (88%)	40/50 (80%)	41/50 (82%)
Adjusted rate	81.1%	90.6%	92.0%	80.6%	83.2%
Terminal rate	18/23 (78%)	36/40 (90%)	32/35 (91%)	28/37 (76%)	33/40 (83%)
First incidence (days)	550	513	570	550	536
Poly-3 test	P=0.303N	P=0.137	P=0.090	P=0.575N	P=0.499
All Organs: Malignant Neoplasms					
Overall rate	24/50 (48%)	9/50 (18%)	11/50 (22%)	9/50 (18%)	10/50 (20%)
Adjusted rate	50.3%	19.0%	23.0%	18.5%	20.7%
Terminal rate	4/23 (17%)	6/40 (15%)	5/35 (14%)	4/37 (11%)	7/40 (18%)
First incidence (days)	571	589	85	611	709
Poly-3 test	P=0.013N	P<0.001N	P=0.004N	P<0.001N	P=0.002N
All Organs: Benign or Malignant Neoplasms					
Overall rate	46/50 (92%)	46/50 (92%)	47/50 (94%)	42/50 (84%)	45/50 (90%)
Adjusted rate	92.0%	92.0%	94.0%	84.0%	91.3%
Terminal rate	19/23 (83%)	36/40 (90%)	32/35 (91%)	29/37 (78%)	37/40 (93%)
First incidence (days)	550	513	85	550	536
Poly-3 test	P=0.360N	P=0.642	P=0.500	P=0.179N	P=0.596N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for clitoral gland, kidney, liver, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidences 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 B4a
Historical Incidence of Renal Tubule Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratory			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	0/50	0/50	0/50
Manganese (II) sulfate monohydrate	0/50	0/50	0/50
Oxazepam	0/50	0/50	0/50
Pentachlorophenol	0/50	0/50	0/50
Primadone	0/50	0/50	0/50
Triamterene	0/50	0/50	0/50
Tricresyl phosphate	0/51	0/51	0/51
Overall Historical Incidence			
Total (%)	0/901	1/901 (0.1%)	1/901 (0.1%)
Mean ± standard deviation		0.1% ± 0.5%	0.1% ± 0.5%
Range		0%-2%	0%-2%

[a] Data as of November 10, 1998

TABLE B4b
Historical Incidence of Urinary Bladder Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Papilloma	Carcinoma	Papilloma or Carcinoma
Historical Incidence at Battelle Columbus Laboratory			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	0/50	0/50	0/50
Manganese (II) sulfate monohydrate	1/49	0/49	1/49
Oxazepam	0/48	0/48	0/48
Pentachlorophenol	0/50	0/50	0/50
Primadone	0/50	0/50	0/50
Triamterene	0/49	0/49	0/49
Tricresyl phosphate	0/51	0/51	0/51
Overall Historical Incidence			
Total (%)	2/891 (0.2%)	0/891	2/891 (0.2%)
Mean ± standard deviation	0.2% ± 0.7%		0.2% ± 0.7%
Range	0%-2%		0%-2%

[a] Data as of November 10, 1998

TABLE B4c
Historical Incidence of Hepatocellular Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratory			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	0/50	0/50	0/50
Manganese (II) sulfate monohydrate	0/50	0/50	0/50
Oxazepam	0/50	0/50	0/50
Pentachlorophenol	0/50	0/50	0/50
Primadone	0/50	0/50	0/50
Triamterene	0/50	0/50	0/50
Tricresyl phosphate	0/51	0/51	0/51
Overall Historical Incidence			
Total (%)	4/901 (0.4%)	0/901	4/901 (0.4%)
Mean ± standard deviation	0.4% ± 1.1%		0.4% ± 1.1%
Range	0%-4%		0%-4%

[a] Data as of November 10, 1998

TABLE B4d
Historical Incidence of Mononuclear Cell Leukemia in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence at Battelle Columbus Laboratory	
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	18/50
Manganese (II) sulfate monohydrate	19/50
Oxazepam	14/50
Pentachlorophenol	15/50
Primadone	13/50
Triamterene	8/50
Tricresyl phosphate	8/51
Overall Historical Incidence	
Total (%)	261/901 (29.0%)
Mean ± standard deviation	29.0% ± 7.8%
Range	16%-42%

[a] Data as of November 10, 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 Anthraquinone[a]

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Disposition Summary					
Animals initially in study	60	50	50	50	60
3-Month interim evaluation	5				5
12-Month interim evaluation	5				5
Early deaths					
Moribund	14	7	12	7	6
Natural deaths	13	3	3	6	4
Survivors					
Terminal sacrifice	23	40	35	37	40
Animals examined microscopically	60	50	50	50	60
3-Month Interim Evaluation					
Alimentary System					
Liver	(5)				(5)
Inflammation	1 (20%)				
Centrilobular, hypertrophy					5 (100%)
Pancreas	(5)				(5)
Inflammation					1 (20%)
Stomach, glandular	(5)				(5)
Erosion					1 (20%)
Cardiovascular System					
Heart	(5)				(5)
Cardiomyopathy					1 (20%)
Endocrine System					
Thyroid gland	(5)				(5)
Follicular cell, hypertrophy					4 (80%)
Genital System					
Ovary	(5)				(5)
Cyst	1 (20%)				
Hematopoietic System					
Spleen	(5)				(5)
Congestion					5 (100%)
Pigmentation					4 (80%)
Respiratory System					
Lung	(5)				(5)
Alveolar epithelium, hyperplasia					1 (20%)
Urinary System					
Kidney	(5)				(5)
Accumulation, hyaline droplet					5 (100%)
Nephropathy	1 (20%)				2 (40%)

[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 Anthraquinone

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
3-Month Interim Evaluation (continued)					
Systems Examined with No Lesions Observed					
General Body System					
Integumentary System					
Musculoskeletal System					
Nervous System					
Special Senses System					
12-Month Interim Evaluation					
Alimentary System					
Intestine large, rectum	(5)				(5)
Parasite metazoan	1 (20%)				
Liver	(5)				(5)
Basophilic focus	3 (60%)				
Hepatodiaphragmatic nodule					1 (20%)
Inflammation	3 (60%)				3 (60%)
Bile duct, hyperplasia					1 (20%)
Centrilobular, hypertrophy					5 (100%)
Pancreas	(5)				(5)
Atrophy	2 (40%)				2 (40%)
Stomach, glandular	(5)				(5)
Erosion	1 (20%)				
Ulcer					1 (20%)
Cardiovascular System					
Heart	(5)				(5)
Cardiomyopathy	3 (60%)				1 (20%)
Inflammation					1 (20%)
Endocrine System					
Adrenal cortex	(5)				(5)
Hyperplasia	1 (20%)				
Pituitary gland	(5)				(5)
Angiectasis	1 (20%)				1 (20%)
Cyst					1 (20%)
Pars distalis, hyperplasia	1 (20%)				1 (20%)
Genital System					
Clitoral gland	(5)				(5)
Inflammation	5 (100%)				5 (100%)
Hematopoietic System					
Bone marrow	(5)				(5)
Hyperplasia					5 (100%)
Lymph node, mandibular	(5)				(5)
Hyperplasia, plasma cell					1 (20%)
Thymus	(5)				(5)
Atrophy					1 (20%)

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
12-Month Interim Evaluation (continued)					
Respiratory System					
Lung	(5)				(5)
Inflammation	5 (100%)				4 (80%)
Trachea	(5)				(5)
Inflammation	1 (20%)				3 (60%)
Urinary System					
Kidney	(5)				(5)
Accumulation, hyaline droplet	2 (40%)				5 (100%)
Nephropathy	3 (60%)				5 (100%)
Medulla, mineralization	1 (20%)				4 (80%)
Urinary bladder	(5)				(5)
Inflammation	2 (40%)				1 (20%)
Systems Examined with No Lesions Observed					
General Body System					
Integumentary System					
Musculoskeletal System					
Nervous System					
Special Senses System					
2-Year Study					
Alimentary System					
Intestine large, colon	(50)	(50)	(49)	(50)	(49)
Parasite metazoan		2 (4%)	2 (4%)	1 (2%)	
Intestine large, rectum	(50)	(50)	(49)	(50)	(50)
Inflammation			1 (2%)		
Parasite metazoan	6 (12%)	11 (22%)	2 (4%)	8 (16%)	
Intestine large, cecum	(50)	(49)	(49)	(50)	(49)
Inflammation				1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)	(49)
Inflammation				1 (2%)	
Intestine small, ileum	(50)	(49)	(49)	(50)	(49)
Cyst			1 (2%)		
Inflammation				1 (2%)	
Liver	(50)	(50)	(50)	(50)	(49)
Angiectasis	3 (6%)	15 (30%)	18 (36%)	15 (30%)	21 (43%)
Basophilic focus	37 (74%)	50 (100%)	34 (68%)	33 (66%)	15 (31%)
Clear cell focus	1 (2%)	3 (6%)	2 (4%)	6 (12%)	3 (6%)
Degeneration, cystic		5 (10%)	10 (20%)	10 (20%)	6 (12%)
Eosinophilic focus	8 (16%)	32 (64%)	34 (68%)	39 (78%)	34 (69%)
Fatty change	1 (2%)				
Fibrosis					1 (2%)
Hematopoietic cell proliferation	3 (6%)	1 (2%)			
Hepatodiaphragmatic nodule	5 (10%)	10 (20%)	7 (14%)	6 (12%)	5 (10%)
Inflammation	25 (50%)	46 (92%)	44 (88%)	38 (76%)	46 (94%)
Mixed cell focus	3 (6%)	30 (60%)	20 (40%)	23 (46%)	13 (27%)
Necrosis	4 (8%)	4 (8%)	5 (10%)	6 (12%)	3 (6%)
Thrombosis	1 (2%)	1 (2%)			
Vacuolization cytoplasmic	5 (10%)	4 (8%)	6 (12%)	4 (8%)	4 (8%)

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Alimentary System (continued)					
Liver (continued)	(50)	(50)	(50)	(50)	(49)
Bile duct, cholangiofibrosis			1 (2%)	1 (2%)	
Bile duct, cyst		1 (2%)		1 (2%)	2 (4%)
Bile duct, hyperplasia	17 (34%)	22 (44%)	20 (40%)	20 (40%)	12 (24%)
Centrilobular, degeneration	1 (2%)	1 (2%)	1 (2%)		1 (2%)
Centrilobular, hypertrophy		18 (36%)	23 (46%)	19 (38%)	26 (53%)
Centrilobular, necrosis				1 (2%)	
Mesentery	(4)	(4)	(6)	(3)	(2)
Inflammation			1 (17%)		
Fat, necrosis	3 (75%)	4 (100%)	4 (67%)	2 (67%)	2 (100%)
Oral mucosa	(1)		(1)		
Gingival, inflammation	1 (100%)				
Pancreas	(50)	(50)	(50)	(50)	(49)
Atrophy	21 (42%)	27 (54%)	16 (32%)	22 (44%)	9 (18%)
Hyperplasia	2 (4%)	1 (2%)		2 (4%)	1 (2%)
Hypertrophy, focal	1 (2%)	2 (4%)			1 (2%)
Metaplasia, hepatocyte				1 (2%)	1 (2%)
Pigmentation			1 (2%)		
Artery, inflammation					1 (2%)
Duct, cyst					1 (2%)
Salivary glands	(50)	(50)	(50)	(50)	(48)
Atrophy		1 (2%)			1 (2%)
Fibrosis		1 (2%)			
Inflammation	1 (2%)				
Stomach, forestomach	(50)	(50)	(50)	(50)	(49)
Edema			1 (2%)		
Erosion	1 (2%)				
Foreign body			1 (2%)		
Hyperplasia	4 (8%)	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Inflammation	1 (2%)	1 (2%)	2 (4%)	1 (2%)	
Necrosis	1 (2%)				
Ulcer	6 (12%)		1 (2%)	3 (6%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)	(49)
Edema			1 (2%)		
Erosion	2 (4%)	1 (2%)	1 (2%)	1 (2%)	
Inflammation					1 (2%)
Mineralization					1 (2%)
Ulcer	2 (4%)	1 (2%)			
Tooth			(1)		
Inflammation			1 (100%)		
Cardiovascular System					
Blood vessel	(50)	(50)	(50)	(50)	(49)
Inflammation	1 (2%)				1 (2%)
Heart	(50)	(50)	(50)	(50)	(48)
Cardiomyopathy	22 (44%)	25 (50%)	27 (54%)	28 (56%)	28 (58%)
Inflammation	2 (4%)	9 (18%)	4 (8%)	1 (2%)	3 (6%)
Artery, inflammation		2 (4%)			
Atrium, thrombosis	2 (4%)				1 (2%)

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Endocrine System					
Adrenal cortex	(50)	(50)	(50)	(50)	(49)
Degeneration, cystic	6 (12%)	2 (4%)	6 (12%)	9 (18%)	3 (6%)
Hematopoietic cell proliferation	1 (2%)				
Hemorrhage				1 (2%)	
Hyperplasia	13 (26%)	12 (24%)	17 (34%)	17 (34%)	3 (6%)
Hypertrophy	3 (6%)	1 (2%)	1 (2%)	7 (14%)	5 (10%)
Mineralization	1 (2%)				
Necrosis					1 (2%)
Vacuolization cytoplasmic	1 (2%)	2 (4%)	1 (2%)	4 (8%)	3 (6%)
Adrenal medulla	(50)	(50)	(50)	(50)	(49)
Cyst					1 (2%)
Hyperplasia	8 (16%)	3 (6%)	4 (8%)	4 (8%)	6 (12%)
Islets, pancreatic	(49)	(50)	(50)	(50)	(49)
Hyperplasia	1 (2%)				
Parathyroid gland	(42)	(44)	(48)	(48)	(41)
Hyperplasia		1 (2%)			1 (2%)
Hyperplasia, focal	1 (2%)				
Pituitary gland	(50)	(50)	(50)	(49)	(49)
Angiectasis	1 (2%)	12 (24%)	2 (4%)	10 (20%)	6 (12%)
Cyst	9 (18%)	7 (14%)	3 (6%)		3 (6%)
Degeneration	1 (2%)				1 (2%)
Pars distalis, hyperplasia	24 (48%)	27 (54%)	21 (42%)	26 (53%)	32 (65%)
Pars intermedia, hyperplasia		1 (2%)			1 (2%)
Pars nervosa, hyperplasia				1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)	(49)
C-cell, hyperplasia	21 (42%)	29 (58%)	20 (40%)	18 (36%)	18 (37%)
Follicle, cyst				1 (2%)	
Follicular cell, hyperplasia				1 (2%)	
General Body System					
None					
Genital System					
Clitoral gland	(49)	(46)	(48)	(49)	(48)
Hyperplasia	2 (4%)	5 (11%)	8 (17%)	4 (8%)	3 (6%)
Inflammation	10 (20%)	21 (46%)	13 (27%)	11 (22%)	12 (25%)
Duct, cyst	4 (8%)	5 (11%)	2 (4%)	4 (8%)	3 (6%)
Ovary	(50)	(50)	(50)	(50)	(49)
Atrophy					1 (2%)
Cyst	5 (10%)	8 (16%)	9 (18%)	8 (16%)	6 (12%)
Granulosa cell, hyperplasia	1 (2%)	1 (2%)			
Interstitial cell, hyperplasia		1 (2%)			
Oviduct		(1)	(1)		
Inflammation		1 (100%)			
Uterus	(50)	(50)	(50)	(50)	(49)
Angiectasis			1 (2%)		
Hemorrhage		1 (2%)	1 (2%)		
Hyperplasia		3 (6%)	3 (6%)	3 (6%)	4 (8%)
Inflammation	1 (2%)				
Cervix, hypertrophy					1 (2%)
Cervix, inflammation			1 (2%)		1 (2%)
Vagina	(1)				(1)
Cyst, squamous					1 (100%)
Inflammation, suppurative	1 (100%)				

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Hematopoietic System					
Bone marrow	(50)	(50)	(50)	(50)	(50)
Atrophy	4 (8%)	13 (26%)	13 (26%)	11 (22%)	13 (26%)
Hyperplasia	19 (38%)	31 (62%)	28 (56%)	19 (38%)	23 (46%)
Infiltration cellular, histiocyte			1 (2%)		
Inflammation					1 (2%)
Lymph node, mandibular	(49)	(49)	(50)	(48)	(47)
Ectasia	2 (4%)		2 (4%)	1 (2%)	
Hyperplasia, plasma cell		1 (2%)	1 (2%)		1 (2%)
Inflammation			1 (2%)	1 (2%)	
Lymph node, mesenteric	(50)	(49)	(49)	(50)	(49)
Atrophy	1 (2%)	1 (2%)			1 (2%)
Ectasia	1 (2%)	1 (2%)	1 (2%)		
Hyperplasia, plasma cell		1 (2%)			
Spleen	(50)	(50)	(50)	(50)	(49)
Congestion	1 (2%)	46 (92%)	42 (84%)	44 (88%)	45 (92%)
Fibrosis	1 (2%)				
Hematopoietic cell proliferation	39 (78%)	50 (100%)	47 (94%)	47 (94%)	46 (94%)
Infarct	1 (2%)			1 (2%)	
Pigmentation	33 (66%)	45 (90%)	48 (96%)	48 (96%)	47 (96%)
Capsule, fibrosis	2 (4%)		1 (2%)	1 (2%)	
Lymphoid follicle, atrophy	1 (2%)		2 (4%)	3 (6%)	
Red pulp, depletion cellular		1 (2%)	2 (4%)	2 (4%)	
Thymus	(46)	(48)	(48)	(49)	(46)
Atrophy	44 (96%)	48 (100%)	47 (98%)	49 (100%)	46 (100%)
Artery, inflammation	1 (2%)				
Integumentary System					
Mammary gland	(50)	(50)	(50)	(50)	(50)
Cyst	5 (10%)	3 (6%)	4 (8%)	2 (4%)	1 (2%)
Hyperplasia	23 (46%)	8 (16%)	11 (22%)	5 (10%)	5 (10%)
Inflammation		1 (2%)			
Skin	(50)	(50)	(50)	(50)	(50)
Acanthosis				1 (2%)	
Cyst epithelial inclusion					1 (2%)
Ulcer	1 (2%)				
Hair follicle, atrophy		1 (2%)			
Subcutaneous tissue, necrosis	1 (2%)				
Musculoskeletal System					
Bone	(50)	(50)	(50)	(50)	(50)
Fibrous osteodystrophy			1 (2%)		
Osteopetrosis	3 (6%)	2 (4%)	3 (6%)	1 (2%)	
Osteoporosis				1 (2%)	
Maxilla, cyst				1 (2%)	
Maxilla, inflammation				1 (2%)	
Nervous System					
Brain	(50)	(50)	(50)	(50)	(49)
Gliosis					1 (2%)
Hemorrhage	1 (2%)	1 (2%)	1 (2%)		
Inflammation	1 (2%)			1 (2%)	1 (2%)
Mineralization					1 (2%)
Thrombosis			1 (2%)		

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

	0 ppm	469 ppm	938 ppm	1,875 ppm	3,750 ppm
2-Year Study (continued)					
Respiratory System					
Lung	(50)	(50)	(50)	(50)	(48)
Foreign body					1 (2%)
Inflammation	13 (26%)	18 (36%)	19 (38%)	21 (42%)	24 (50%)
Pigmentation	44 (88%)	50 (100%)	48 (96%)	47 (94%)	48 (100%)
Thrombosis	1 (2%)				
Alveolar epithelium, hyperplasia	3 (6%)	7 (14%)	2 (4%)	5 (10%)	
Nose	(50)	(50)	(50)	(50)	(50)
Foreign body	4 (8%)	1 (2%)	1 (2%)		1 (2%)
Inflammation	4 (8%)	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Nasolacrimal duct, inflammation	1 (2%)				
Respiratory epithelium, inflammation			1 (2%)		
Special Senses System					
Eye	(2)	(3)	(2)	(3)	(5)
Cataract	2 (100%)	3 (100%)	1 (50%)	2 (67%)	3 (60%)
Degeneration			1 (50%)	2 (67%)	2 (40%)
Hemorrhage					1 (20%)
Inflammation			1 (50%)		
Cornea, inflammation	1 (50%)				
Lens, mineralization		2 (67%)	1 (50%)		
Retina, degeneration	1 (50%)	3 (100%)	2 (100%)	1 (33%)	3 (60%)
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(49)
Accumulation, hyaline droplet	33 (66%)	48 (96%)	45 (90%)	44 (88%)	44 (90%)
Hydronephrosis			2 (4%)		1 (2%)
Infarct	1 (2%)		1 (2%)		
Inflammation	3 (6%)				
Nephropathy	39 (78%)	49 (98%)	47 (94%)	49 (98%)	49 (100%)
Pigmentation	27 (54%)	50 (100%)	48 (96%)	50 (100%)	47 (96%)
Medulla, mineralization	17 (34%)	25 (50%)	27 (54%)	28 (56%)	20 (41%)
Pelvis, calculus, microscopic observation only	1 (2%)				
Pelvis, inflammation		1 (2%)	1 (2%)		
Renal tubule, hyperplasia		12 (24%)	13 (26%)	15 (30%)	11 (22%)
Renal tubule, hyperplasia, oncocytic		2 (4%)		1 (2%)	1 (2%)
Transitional epithelium, hyperplasia		5 (10%)	12 (24%)	3 (6%)	10 (20%)
Urinary bladder	(49)	(49)	(49)	(50)	(49)
Calculus, microscopic observation only				1 (2%)	
Inflammation	13 (27%)	16 (33%)	9 (18%)	25 (50%)	17 (35%)
Transitional epithelium, hyperplasia		1 (2%)	1 (2%)	4 (8%)	4 (8%)
Transitional epithelium, metaplasia, squamous				1 (2%)	

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

TABLE C1 Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Anthraquinone C-3
TABLE C2 Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone C-6
TABLE C3 Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Anthraquinone C-22
TABLE C4a Historical Incidence of Liver Neoplasms in Untreated Male $B6C3F_1$ Mice C-25
TABLE C4b Historical Incidence of Thyroid Gland Follicular Cell Adenoma in Untreated Male $B6C3F_1$ Mice C-26
TABLE C5 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Anthraquinone C-27

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	3	3	3	8
Natural deaths	2	6	4	19
Survivors				
Terminal sacrifice	45	41	43	23
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Liver	(50)	(50)	(50)	(49)
Hemangiosarcoma	1 (2%)			1 (2%)
Hepatoblastoma	1 (2%)	5 (10%)	11 (22%)	21 (43%)
Hepatoblastoma, multiple		1 (2%)		16 (33%)
Hepatocellular carcinoma	7 (14%)	9 (18%)	12 (24%)	12 (24%)
Hepatocellular carcinoma, multiple	1 (2%)	4 (8%)	5 (10%)	9 (18%)
Hepatocellular adenoma	16 (32%)	10 (20%)	10 (20%)	10 (20%)
Hepatocellular adenoma, multiple	5 (10%)	22 (44%)	28 (56%)	31 (63%)
Hepatocholangiocarcinoma		1 (2%)		
Histiocytic sarcoma		1 (2%)	2 (4%)	1 (2%)
Mesentery	(2)	(2)	(1)	(5)
Oral mucosa				(1)
Squamous cell carcinoma				1 (100%)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Capsule, adenoma				1 (2%)
Adrenal medulla	(49)	(50)	(50)	(49)
Pheochromocytoma benign	1 (2%)		1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(42)
Adenoma	1 (2%)		1 (2%)	
Carcinoma	1 (2%)			
Pituitary gland	(48)	(48)	(47)	(46)
Pars distalis, adenoma			1 (2%)	
Thyroid gland	(50)	(50)	(49)	(46)
C-cell, carcinoma				1 (2%)
Follicular cell, adenoma			2 (4%)	2 (4%)
General Body System				
None				

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Testes	(50)	(49)	(50)	(50)
Interstitial cell, adenoma		1 (2%)	1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			
Lymph node	(1)	(1)	(3)	
Mediastinal, histiocytic sarcoma			1 (33%)	
Lymph node, mandibular	(48)	(45)	(44)	(48)
Lymph node, mesenteric	(49)	(49)	(47)	(40)
Histiocytic sarcoma			1 (2%)	
Spleen	(50)	(50)	(49)	(42)
Hemangiosarcoma	2 (4%)			
Histiocytic sarcoma			1 (2%)	1 (2%)
Thymus	(45)	(48)	(40)	(42)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Integumentary System				
Skin	(50)	(50)	(50)	(49)
Subcutaneous tissue, hemangiosarcoma				1 (2%)
Subcutaneous tissue, schwannoma malignant			2 (4%)	
Musculoskeletal System				
Skeletal muscle			(1)	
Schwannoma malignant, metastatic, skin			1 (100%)	
Nervous System				
None				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	10 (20%)	7 (14%)	6 (12%)	8 (16%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)	1 (2%)		
Alveolar/bronchiolar carcinoma	8 (16%)	11 (22%)	8 (16%)	3 (6%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)	3 (6%)	1 (2%)
Hepatoblastoma, metastatic, liver			1 (2%)	8 (16%)
Hepatocellular carcinoma, metastatic, liver	4 (8%)	1 (2%)	2 (4%)	5 (10%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Histiocytic sarcoma		1 (2%)	2 (4%)	
Special Senses System				
Harderian gland	(3)	(4)	(4)	(4)
Adenoma	3 (100%)	4 (100%)	4 (100%)	4 (100%)

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(47)
Histiocytic sarcoma			1 (2%)	
Renal tubule, adenoma		1 (2%)	2 (4%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	2 (4%)	1 (2%)
Lymphoma malignant	3 (6%)	3 (6%)	2 (4%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	35	38	47	48
Total primary neoplasms	62	82	102	126
Total animals with benign neoplasms	27	34	41	43
Total benign neoplasms	37	46	56	57
Total animals with malignant neoplasms	18	28	33	45
Total malignant neoplasms	25	36	46	69
Total animals with metastatic neoplasms	4	2	4	12
Total metastatic neoplasms	4	4	4	13

[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 neoplams except metastatic neoplasms

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

Number of Days on Study	015	550	662	702	710	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730
Carcass ID Number	009	023	034	026	013	001	005	010	011	017	018	020	022	024	025	029	031	033	038	039	041	042	002	003	004
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Hepatoblastoma																									
Hepatocellular carcinoma				X						X				X						X					X
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma			X	X			X							X			X		X					X	
Hepatocellular adenoma, multiple																		X							X
Mesentery																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth							+			+							+	+	+						
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Pheochromocytoma benign													X												
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X													
Carcinoma																									
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	M	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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 Anthraquinone: 0 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	
Carcass ID Number	006	007	008	014	016	019	028	030	032	035	036	043	044	045	048	049	012	015	021	027	037	040	046	047	050	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Hepatoblastoma				X																						1
Hepatocellular carcinoma											X														X	7
Hepatocellular carcinoma, multiple		X																								1
Hepatocellular adenoma	X							X			X	X	X			X	X	X						X		16
Hepatocellular adenoma, multiple									X	X									X							5
Mesentery				+																		+				2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth				+	+	+								+	+		+		+	+		+				14
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Carcinoma																			X							1
Parathyroid gland	M	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	M	+	+	41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	48
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 Anthraquinone: 0 ppm

Number of Days on Study	015	550	662	702	710	729	229	229	229	229	229	229	229	229	229	229	229	229	229	229	229	229	730	730	730
Carcass ID Number	009	023	034	026	013	001	005	010	011	017	018	020	022	024	025	029	031	033	038	039	041	042	022	023	024
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Lymph node									+																
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X					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	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma			X	X	X	X	X						X				X								
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma										X										X				X	
Hepatocellular carcinoma, metastatic, liver														X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye			+																						
Harderian gland		+	+																						
Adenoma		X	X																						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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 Anthraquinone: 0 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	
Carcass ID Number	006	007	008	014	016	019	028	030	032	035	036	043	044	045	048	049	012	015	021	027	037	040	046	047	050	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Lymph node																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										2
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	45
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
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X															X			X							10
Alveolar/bronchiolar adenoma, multiple					X																					1
Alveolar/bronchiolar carcinoma	X			X					X							X							X			8
Hepatocellular carcinoma, metastatic, liver		X									X														X	4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										1
Harderian gland																								+		3
Adenoma																								X		3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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 Anthraquinone: 833 ppm

Number of Days on Study	456	464	601	657	658	662	666	667	710	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	098	058	077	080	093	010	066	088	053	052	054	056	057	059	062	064	067	068	072	073	074	082	083	084	086
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma					X																				
Hepatoblastoma, multiple																									
Hepatocellular carcinoma		X	X													X					X		X		
Hepatocellular carcinoma, multiple											X														
Hepatocellular adenoma										X	X					X						X		X	
Hepatocellular adenoma, multiple								X	X			X		X			X				X		X		X
Hepatocholangiocarcinoma								X																	
Histiocytic sarcoma																		X							
Mesentery							+																		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth					+				+			+								+					
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver								X																	
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Interstitial cell, adenoma																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 833 ppm

Number of Days on Study	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	
Carcass ID Number	087	089	094	096	099	051	055	060	063	065	069	070	071	075	076	079	090	091	092	095	097	061	078	081	085	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
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
Hepatoblastoma	X								X											X		X				5
Hepatoblastoma, multiple								X																		1
Hepatocellular carcinoma							X						X	X		X										9
Hepatocellular carcinoma, multiple				X					X												X					4
Hepatocellular adenoma			X						X							X						X		X		10
Hepatocellular adenoma, multiple	X	X		X			X	X		X		X	X	X	X					X	X		X		X	22
Hepatocholangiocarcinoma																										1
Histiocytic sarcoma																										1
Mesentery										+																2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+	+		+		+	+		+			+	+	+	+					+	+	+	+		+	19
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocholangiocarcinoma, metastatic, liver																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	M	M	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	M	39
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Interstitial cell, adenoma																								X		1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 833 ppm

Number of Days on Study	456	464	601	657	658	662	666	667	710	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729
Carcass ID Number	098	058	077	080	093	100	066	088	053	052	054	056	057	059	062	064	067	068	072	073	074	082	083	084	086
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node																									
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocholangiocarcinoma, metastatic, liver								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
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																	X								
Alveolar/bronchiolar adenoma, multiple																									X
Alveolar/bronchiolar carcinoma			X								X	X						X				X	X		
Alveolar/bronchiolar carcinoma, multiple																									
Hepatocellular carcinoma, metastatic, liver											X														
Hepatocholangiocarcinoma, metastatic, liver								X																	
Histiocytic sarcoma																		X							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																		+							
Harderian gland						+												+							
Adenoma						X												X							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																					X				
Urinary bladder	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																		X							
Lymphoma malignant														X											

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 833 ppm

Number of Days on Study	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	
Carcass ID Number	087	089	094	096	099	051	055	060	063	065	069	070	071	075	076	079	090	091	092	095	097	061	078	081	085	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node							+																			1
Lymph node, mandibular	+	+	M	+	+	+	M	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	45
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocholangiocarcinoma, metastatic, liver																										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
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X	X					X	X						X					X						7
Alveolar/bronchiolar adenoma, multiple																										1
Alveolar/bronchiolar carcinoma		X									X											X	X	X		11
Alveolar/bronchiolar carcinoma, multiple												X														1
Hepatocellular carcinoma, metastatic, liver																										1
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye								+																		2
Harderian gland								+					+													4
Adenoma								X					X													4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant							X				X															3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 2,500 ppm

Number of Days on Study	453	481	491	662	685	711	727	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730
Carcass ID Number	145	101	126	121	104	134	102	103	109	110	112	114	115	116	122	125	129	132	136	138	139	144	150	106	107
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma		X								X		X			X								X		
Hepatocellular carcinoma								X			X					X						X		X	
Hepatocellular carcinoma, multiple															X		X								
Hepatocellular adenoma				X					X	X		X					X	X	X			X	X		
Hepatocellular adenoma, multiple						X					X		X	X	X	X				X	X				
Histiocytic sarcoma					X																	X			
Mesentery																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth						+					+	+	+				+		+						
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																				X					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X																		
Parathyroid gland	M	+	M	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	M	M	+	+	+	+	+
Pituitary gland	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma								X																	
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma										X															
Penis																									
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 2,500 ppm

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	
Carcass ID Number	108	113	118	119	120	123	124	127	128	130	135	137	141	142	143	147	149	105	111	117	131	133	140	146	148	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	47
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
Hepatoblastoma	X	X		X													X			X	X					11
Hepatocellular carcinoma							X		X						X	X			X					X	X	12
Hepatocellular carcinoma, multiple												X						X					X			5
Hepatocellular adenoma						X																				10
Hepatocellular adenoma, multiple	X	X	X	X			X	X	X	X	X	X		X	X		X	X	X		X	X	X	X	X	28
Histiocytic sarcoma																										2
Mesentery	+																									1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+			+		+					+	+						+			+	+				14
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	M	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	47
Pars distalis, adenoma										X																1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell, adenoma									X																	2
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Penis															+											1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	49
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																						X				1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 2,500 ppm

Number of Days on Study	453	481	491	662	685	711	727	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730
Carcass ID Number	145	101	126	121	104	134	102	103	109	110	112	114	115	116	122	125	129	132	136	138	139	144	150	106	107
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+																					+			
Mediastinal, histiocytic sarcoma																						X			
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+
Lymph node, mesenteric	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																						X			
Spleen	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																						X			
Thymus	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
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, schwannoma malignant	X																								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
Schwannoma malignant, metastatic, skin	X																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve							+																		
Spinal cord							+																		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X												X	X			X
Alveolar/bronchiolar carcinoma							X			X										X				X	
Alveolar/bronchiolar carcinoma, multiple						X											X								
Hepatoblastoma, metastatic, liver		X																							
Hepatocellular carcinoma, metastatic, liver																X	X								
Histiocytic sarcoma					X																	X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland				+																					
Adenoma				X																					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																						X			
Renal tubule, adenoma									X																
Urinary bladder	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma					X																	X			
Lymphoma malignant																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 2,500 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	731	
Carcass ID Number	108	113	118	119	120	123	124	127	128	130	135	137	141	142	143	147	149	105	111	117	131	133	140	146	148	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+																									3
Mediastinal, histiocytic sarcoma																										1
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	47
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	M	M	M	+	+	+	+	+	+	+	M	+	M	+	+	+	M	+	+	40
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, schwannoma malignant				X																						2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Schwannoma malignant, metastatic, skin																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X															X									6
Alveolar/bronchiolar carcinoma																			X		X			X	X	8
Alveolar/bronchiolar carcinoma, multiple												X														3
Hepatoblastoma, metastatic, liver																										1
Hepatocellular carcinoma, metastatic, liver																										2
Histiocytic sarcoma																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland	+							+														+				4
Adenoma	X							X														X				4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Renal tubule, adenoma				X																						2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymphoma malignant	X							X																		2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 7,500 ppm

Number of Days on Study	456	490	497	540	554	559	569	570	586	590	617	621	621	623	638	638	642	647	647	658	664	687	690	690	702
Carcass ID Number	181	163	167	170	190	199	159	185	178	152	166	186	193	179	153	157	188	171	175	164	198	158	172	191	165
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M
Liver	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																		X							
Hepatoblastoma		X					X	X				X	X		X		X	X						X	X
Hepatoblastoma, multiple				X	X					X	X					X			X	X	X	X	X		
Hepatocellular carcinoma			X											X				X					X		
Hepatocellular carcinoma, multiple	X														X					X					
Hepatocellular adenoma		X		X										X		X	X					X			
Hepatocellular adenoma, multiple					X		X	X		X		X	X		X				X				X	X	X
Histiocytic sarcoma														X											
Mesentery									+									+				+		+	
Oral mucosa								+																	
Squamous cell carcinoma								X																	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth															+										
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																									
Adrenal medulla	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	A	A	+	A	+	A	+	+	+	+	A	I	+	+	A	+	+	+	+	+	I	+	+	+	+
Parathyroid gland	M	+	+	+	+	M	+	+	+	M	+	M	M	M	M	+	+	M	M	+	+	+	M	M	+
Pituitary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	A	+	A	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, carcinoma																							X		
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 7,500 ppm

Number of Days on Study	707	708	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	
Carcass ID Number	161	182	162	180	184	187	192	195	197	160	168	169	173	177	183	194	200	151	154	155	156	174	176	189	196	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma			X																							1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Hepatoblastoma		X			X		X		X					X				X	X	X	X		X	X		21
Hepatoblastoma, multiple	X									X	X						X					X			X	16
Hepatocellular carcinoma			X					X	X		X	X								X	X	X				12
Hepatocellular carcinoma, multiple					X		X						X		X				X					X		9
Hepatocellular adenoma		X		X												X	X									10
Hepatocellular adenoma, multiple	X				X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	31
Histiocytic sarcoma																										1
Mesentery																	+									5
Oral mucosa																										1
Squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																									X	1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth								+		+	+												+			5
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma													X													1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																	X									1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	37
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
C-cell, carcinoma																										1
Follicular cell, adenoma							X															X				2
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 7,500 ppm

Number of Days on Study	456	490	497	540	554	559	569	570	586	590	617	621	621	623	638	638	642	647	647	658	664	687	690	690	702
Carcass ID Number	181	163	167	170	190	199	159	185	178	152	166	186	193	179	153	157	188	171	175	164	198	158	172	191	165
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	M	M	+	M	M	+	M	+
Spleen	+	M	+	A	+	A	+	+	A	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma														X											
Thymus	M	+	+	+	+	+	M	+	+	M	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																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma		X										X									X				
Alveolar/bronchiolar carcinoma																									
Alveolar/bronchiolar carcinoma, multiple																									
Hepatoblastoma, metastatic, liver							X				X	X										X		X	
Hepatocellular carcinoma, metastatic, liver			X																	X			X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland																		+	+						
Adenoma																		X	X						
Urinary System																									
Kidney	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	A	A	+	+	A	+	+	+	+	A	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma														X											

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Anthraquinone: 7,500 ppm

Number of Days on Study	707	708	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	
Carcass ID Number	161	182	162	180	184	187	192	195	197	160	168	169	173	177	183	194	200	151	154	155	156	174	176	189	196	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	40
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	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	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Subcutaneous tissue, hemangiosarcoma															X											1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X		X		X					X			X										8
Alveolar/bronchiolar carcinoma					X				X														X			3
Alveolar/bronchiolar carcinoma, multiple																			X							1
Hepatoblastoma, metastatic, liver										X								X				X				8
Hepatocellular carcinoma, metastatic, liver									X													X				5
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland													+			+										4
Adenoma													X			X										4
Urinary System																										
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Harderian Gland: Adenoma				
Overall rate[a]	3/50 (6%)	4/50 (8%)	4/50 (8%)	4/50 (8%)
Adjusted rate[b]	6.1%	8.5%	8.4%	9.9%
Terminal rate[c]	1/45 (2%)	3/41 (7%)	3/43 (7%)	2/23 (9%)
First incidence (days)	550	662	662	647
Poly-3 test[d]	P=0.375	P=0.480	P=0.486	P=0.398
Kidney: Renal Tubule Adenoma (Step Sections)				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/48 (4%)
Adjusted rate	2.1%	2.1%	6.3%	5.2%
Terminal rate	1/45 (2%)	1/41 (2%)	3/43 (7%)	1/23 (4%)
First incidence (days)	729 (T)	729 (T)	729 (T)	707
Poly-3 test	P=0.302	P=0.755	P=0.301	P=0.427
Kidney: Renal Tubule Adenoma (Original and Step Sections)				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	2/48 (4%)
Adjusted rate	2.1%	4.3%	8.5%	5.2%
Terminal rate	1/45 (2%)	2/41 (5%)	4/43 (9%)	1/23 (4%)
First incidence (days)	729 (T)	729 (T)	729 (T)	707
Poly-3 test	P=0.385	P=0.491	P=0.175	P=0.427
Liver: Hepatocellular Adenoma				
Overall rate	21/50 (42%)	32/50 (64%)	38/50 (76%)	41/49 (84%)
Adjusted rate	43.4%	68.0%	79.7%	89.4%
Terminal rate	19/45 (42%)	30/41 (73%)	36/43 (84%)	22/23 (96%)
First incidence (days)	662	677	662	490
Poly-3 test	P<0.001	P=0.011	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rate	8/50 (16%)	13/50 (26%)	17/50 (34%)	21/49 (43%)
Adjusted rate	16.6%	27.1%	35.9%	49.8%
Terminal rate	7/45 (16%)	11/41 (27%)	17/43 (40%)	14/23 (61%)
First incidence (days)	702	464	729 (T)	456
Poly-3 test	P<0.001	P=0.160	P=0.026	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	25/50 (50%)	34/50 (68%)	41/50 (82%)	46/49 (94%)
Adjusted rate	51.7%	70.5%	86.0%	96.1%
Terminal rate	23/45 (51%)	30/41 (73%)	39/43 (91%)	23/23 (100%)
First incidence (days)	662	464	662	456
Poly-3 test	P<0.001	P=0.043	P<0.001	P<0.001
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	6/50 (12%)	11/50 (22%)	37/49 (76%)
Adjusted rate	2.1%	12.8%	22.9%	79.2%
Terminal rate	1/45 (2%)	5/41 (12%)	10/43 (23%)	15/23 (65%)
First incidence (days)	729 (T)	658	481	490
Poly-3 test	P<0.001	P=0.053	P=0.002	P<0.001

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	9/50 (18%)	18/50 (36%)	27/50 (54%)	45/49 (92%)
Adjusted rate	18.7%	37.3%	56.2%	92.7%
Terminal rate	8/45 (18%)	15/41 (37%)	26/43 (61%)	20/23 (87%)
First incidence (days)	702	464	481	456
Poly-3 test	P<0.001	P=0.033	P<0.001	P<0.001
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	26/50 (52%)	35/50 (70%)	43/50 (86%)	48/49 (98%)
Adjusted rate	53.8%	72.2%	88.9%	98.9%
Terminal rate	24/45 (53%)	30/41 (73%)	40/43 (93%)	23/23 (100%)
First incidence (days)	662	464	481	456
Poly-3 test	P<0.001	P=0.045	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	11/50 (22%)	8/50 (16%)	6/50 (12%)	8/50 (16%)
Adjusted rate	22.7%	17.1%	12.7%	19.5%
Terminal rate	8/45 (18%)	8/41 (20%)	6/43 (14%)	5/23 (22%)
First incidence (days)	662	729 (T)	729 (T)	490
Poly-3 test	P=0.489N	P=0.335N	P=0.155N	P=0.454N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	8/50 (16%)	12/50 (24%)	11/50 (22%)	4/50 (8%)
Adjusted rate	16.7%	25.4%	23.2%	10.1%
Terminal rate	8/45 (18%)	11/41 (27%)	9/43 (21%)	4/23 (17%)
First incidence (days)	729 (T)	601	711	729 (T)
Poly-3 test	P=0.138N	P=0.213	P=0.295	P=0.281N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	17/50 (34%)	19/50 (38%)	17/50 (34%)	12/50 (24%)
Adjusted rate	35.1%	40.2%	35.9%	29.2%
Terminal rate	14/45 (31%)	18/41 (44%)	15/43 (35%)	9/23 (39%)
First incidence (days)	662	601	711	490
Poly-3 test	P=0.235N	P=0.379	P=0.554	P=0.356N
All Organs: Hemangiosarcoma				
Overall rate	2/50 (4%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.2%	0.0%	2.1%	7.5%
Terminal rate	1/45 (2%)	0/41 (0%)	1/43 (2%)	2/23 (9%)
First incidence (days)	710	—[e]	729 (T)	647
Poly-3 test	P=0.151	P=0.244N	P=0.505N	P=0.418
All Organs: Malignant Lymphoma				
Overall rate	3/50 (6%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	6.3%	6.4%	4.2%	0.0%
Terminal rate	3/45 (7%)	3/41 (7%)	2/43 (5%)	0/23 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	—
Poly-3 test	P=0.104N	P=0.650	P=0.506N	P=0.156N

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
All Organs: Benign Neoplasms				
Overall rate	27/50 (54%)	34/50 (68%)	41/50 (82%)	43/50 (86%)
Adjusted rate	55.1%	71.9%	86.0%	91.7%
Terminal rate	23/45 (51%)	31/41 (76%)	38/43 (88%)	22/23 (96%)
First incidence (days)	550	662	662	490
Poly-3 test	P<0.001	P=0.064	P<0.001	P<0.001
All Organs: Malignant Neoplasms				
Overall rate	18/50 (36%)	28/50 (56%)	33/50 (66%)	45/50 (90%)
Adjusted rate	37.4%	57.8%	67.3%	91.9%
Terminal rate	16/45 (36%)	24/41 (59%)	28/43 (65%)	20/23 (87%)
First incidence (days)	702	464	453	456
Poly-3 test	P<0.001	P=0.033	P=0.002	P<0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	35/50 (70%)	38/50 (76%)	47/50 (94%)	48/50 (96%)
Adjusted rate	71.4%	78.0%	95.3%	98.0%
Terminal rate	31/45 (69%)	32/41 (78%)	41/43 (95%)	23/23 (100%)
First incidence (days)	550	464	453	456
Poly-3 test	P<0.001	P=0.305	P<0.001	P<0.001

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for kidney, liver and lung; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidences 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 C4a
Historical Incidence of Liver Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	17/50	11/50	25/50
Manganese (II) sulfate monohydrate	30/50	9/50	34/50
Oxazepam	17/49	9/49	23/49
Primadone	22/50	12/50	31/50
Triamterene	17/50	5/50	20/50
Triamterene	21/50	9/50	25/50
Tricresyl phosphate	18/52	15/52	28/52
Overall Historical Incidence			
Total (%)	333/850 (39.2%)	166/850 (19.5%)	440/850 (51.8%)
Mean ± standard deviation	39.2% ± 10.1%	19.5% ± 5.0%	51.8% ± 8.3%
Range	20%-60%	10%-29%	40%-68%
	Hepatoblastoma	**Hepatocellular Carcinoma or Hepatoblastoma**	**Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma**
Historical Incidence at Battelle Columbus Laboratories			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	0/50	11/50	25/50
Manganese (II) sulfate monohydrate	0/50	9/50	34/50
Oxazepam	0/49	9/49	23/49
Primadone	0/50	12/50	31/50
Triamterene	0/50	5/50	20/50
Triamterene	0/50	9/50	25/50
Tricresyl phosphate	0/52	15/52	28/52
Overall Historical Incidence			
Total (%)	0/850	166/850 (19.5%)	440/850 (51.8%)
Mean ± standard deviation		19.5% ± 5.0%	51.8% ± 8.3%
Range		10%-29%	40%-68%

[a] Data as of November 3, 1998

TABLE C4b
Historical Incidence of Thyroid Gland Follicular Cell Adenoma in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls
Historical Incidence at Battelle Columbus Laboratory	
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	0/50
Manganese (II) sulfate monohydrate	0/50
Oxazepam	0/49
Pentachlorophenol	0/49
Primadone	0/50
Triamterene	1/50
Tricresyl phosphate	0/52
Overall Historical Incidence	
Total (%)	12/846 (1.4%)
Mean ± standard deviation	1.4% ± 1.6%
Range	0%-4%

[a] Data as of November 3, 1998

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	3	3	3	8
Natural deaths	2	6	4	19
Survivors				
Terminal sacrifice	45	41	43	23
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(50)	(48)	(47)	(48)
Cyst			1 (2%)	
Liver	(50)	(50)	(50)	(49)
Basophilic focus		3 (6%)	1 (2%)	
Clear cell focus	14 (28%)	12 (24%)	9 (18%)	2 (4%)
Degeneration, fatty, focal		7 (14%)	6 (12%)	
Eosinophilic focus	14 (28%)	17 (34%)	24 (48%)	20 (41%)
Fatty change, focal	1 (2%)			1 (2%)
Hematopoietic cell proliferation		2 (4%)		4 (8%)
Infarct				1 (2%)
Inflammation, granulomatous		1 (2%)		
Mineralization				2 (4%)
Mixed cell focus	3 (6%)	2 (4%)	4 (8%)	1 (2%)
Necrosis, focal	2 (4%)	3 (6%)	3 (6%)	8 (16%)
Bile duct, cyst	1 (2%)	2 (4%)		1 (2%)
Centrilobular, degeneration, fatty			1 (2%)	
Centrilobular, hypertrophy	24 (48%)	34 (68%)	41 (82%)	33 (67%)
Hepatocyte, erythrophagocytosis	1 (2%)	9 (18%)	13 (26%)	6 (12%)
Mesentery	(2)	(2)	(1)	(5)
Fat, necrosis	2 (100%)	2 (100%)		5 (100%)
Pancreas	(50)	(50)	(50)	(50)
Inflammation, acute		1 (2%)		
Acinus, atrophy	2 (4%)	1 (2%)		1 (2%)
Duct, necrosis		1 (2%)		
Stomach, forestomach	(50)	(49)	(50)	(50)
Cyst			1 (2%)	
Ulcer		1 (2%)		2 (4%)
Epithelium, hyperplasia, focal				2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Foreign body		1 (2%)		
Inflammation, focal, suppurative		1 (2%)		
Metaplasia, squamous	1 (2%)			
Mineralization				1 (2%)
Ulcer	2 (4%)			1 (2%)
Epithelium, hyperplasia, focal	1 (2%)			
Tooth	(14)	(19)	(14)	(5)
Inflammation, suppurative		1 (5%)	1 (7%)	
Malformation	14 (100%)	19 (100%)	14 (100%)	5 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Atrium, thrombosis	2 (4%)			
Myocardium, degeneration	4 (8%)		3 (6%)	5 (10%)
Myocardium, mineralization			1 (2%)	1 (2%)
Valve, fibrosis	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 Anthraquinone

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hyperplasia, focal	8 (16%)	8 (16%)	8 (16%)	5 (10%)
Subcapsular, hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	
Adrenal medulla	(49)	(50)	(50)	(49)
Hyperplasia, focal	2 (4%)			
Islets, pancreatic	(50)	(50)	(50)	(42)
Hyperplasia	40 (80%)	40 (80%)	29 (58%)	17 (40%)
Pituitary gland	(48)	(48)	(47)	(46)
Pars distalis, hyperplasia, focal	1 (2%)		2 (4%)	
Thyroid gland	(50)	(50)	(49)	(46)
Follicular cell, hyperplasia	7 (14%)	10 (20%)	15 (31%)	21 (46%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm				3 (6%)
Hemorrhage			1 (2%)	
Preputial gland	(50)	(50)	(49)	(49)
Cyst	22 (44%)	25 (50%)	32 (65%)	22 (45%)
Inflammation, granulomatous		4 (8%)		1 (2%)
Inflammation, suppurative	7 (14%)	4 (8%)	4 (8%)	4 (8%)
Prostate	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation	1 (2%)			
Inflammation, chronic	7 (14%)	3 (6%)	4 (8%)	
Testes	(50)	(49)	(50)	(50)
Germinal epithelium, degeneration			2 (4%)	3 (6%)
Germinal epithelium, mineralization	2 (4%)		1 (2%)	1 (2%)
Hematopoietic System				
Lymph node	(1)	(1)	(3)	
Hyperplasia, lymphoid		1 (100%)	1 (33%)	
Lymph node, mesenteric	(49)	(49)	(47)	(40)
Angiectasis				1 (3%)
Erythrophagocytosis		1 (2%)		
Hyperplasia, lymphoid	1 (2%)			
Spleen	(50)	(50)	(49)	(42)
Atrophy	1 (2%)	1 (2%)		
Hematopoietic cell proliferation	12 (24%)	14 (28%)	12 (24%)	30 (71%)
Infiltration cellular, mast cell	1 (2%)			
Pigmentation			1 (2%)	1 (2%)
Lymphoid follicle, atrophy				1 (2%)
Lymphoid follicle, hyperplasia	4 (8%)		1 (2%)	
Integumentary System				
Skin	(50)	(50)	(50)	(49)
Ulcer		1 (2%)	1 (2%)	
Conjunctiva, inflammation, chronic			1 (2%)	
Prepuce, hemorrhage	1 (2%)			
Subcutaneous tissue, edema	1 (2%)	1 (2%)		

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Integumentary System (continued)				
Skin (continued)	(50)	(50)	(50)	(49)
Subcutaneous tissue, inflammation, chronic active			1 (2%)	
Subcutaneous tissue, inflammation, suppurative	1 (2%)			
Subcutaneous tissue, mineralization	1 (2%)			
Musculoskeletal System				
None				
Nervous System				
Brain	(50)	(50)	(50)	(50)
Meninges, infiltration cellular, lymphocyte	1 (2%)			
Peripheral nerve			(1)	
Axon, degeneration			1 (100%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Inflammation, chronic		1 (2%)		
Inflammation, granulomatous			1 (2%)	
Alveolar epithelium, hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	
Vein, thrombosis		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Trachea	(50)	(50)	(50)	(50)
Glands, hyperplasia		1 (2%)		
Special Senses System				
Eye	(1)	(2)		
Degeneration		1 (50%)		
Cornea, inflammation, chronic	1 (100%)	1 (50%)		
Urinary System				
Kidney	(50)	(49)	(49)	(47)
Degeneration	1 (2%)			
Infarct	1 (2%)			
Metaplasia, osseous			1 (2%)	
Necrosis		1 (2%)		
Nephropathy	29 (58%)	25 (50%)	27 (55%)	18 (38%)
Pigmentation		2 (4%)	2 (4%)	18 (38%)
Glomerulus, inflammation, chronic	1 (2%)			
Pelvis, inflammation, suppurative		1 (2%)		
Renal tubule, cyst	2 (4%)			
Renal tubule, hyperplasia, focal		1 (2%)	1 (2%)	
Renal tubule, pigmentation, lipofuscin				1 (2%)
Renal tubule, vacuolization cytoplasmic				1 (2%)
Urinary bladder	(50)	(49)	(49)	(45)
Calculus gross observation		1 (2%)		
Transitional epithelium, inclusion body, intracytoplasmic		46 (94%)	46 (94%)	42 (93%)

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

TABLE D1 Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Anthraquinone D-3
TABLE D2 Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone D-6
TABLE D3 Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Anthraquinone D-22
TABLE D4a Historical Incidence of Liver Neoplasms in Untreated Female $B6C3F_1$ Mice D-25
TABLE D4b Historical Incidence of Thyroid Gland Neoplasms in Untreated Female $B6C3F_1$ Mice . . D-26
TABLE D5 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Anthraquinone D-27

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			3	
Moribund	6	3	4	2
Natural deaths	9	5	8	5
Survivors				
Terminal sacrifice	35	42	35	42
Missing				1
Animals examined microscopically	50	50	50	49
Alimentary System				
Intestine small, jejunum	(50)	(50)	(50)	(49)
Liver	(49)	(50)	(50)	(49)
Hemangiosarcoma		1 (2%)		
Hepatoblastoma				1 (2%)
Hepatocellular carcinoma	2 (4%)	1 (2%)	7 (14%)	6 (12%)
Hepatocellular carcinoma, multiple		2 (4%)	1 (2%)	2 (4%)
Hepatocellular adenoma	5 (10%)	11 (22%)	14 (28%)	10 (20%)
Hepatocellular adenoma, multiple	1 (2%)	17 (34%)	13 (26%)	30 (61%)
Histiocytic sarcoma	2 (4%)	1 (2%)	2 (4%)	
Ito cell tumor malignant	1 (2%)			
Mesentery	(6)	(4)	(6)	(7)
Histiocytic sarcoma			1 (17%)	
Pancreas	(50)	(50)	(50)	(49)
Salivary glands	(49)	(49)	(50)	(49)
Stomach, forestomach	(50)	(50)	(50)	(49)
Squamous cell papilloma	3 (6%)			1 (2%)
Stomach, glandular	(50)	(50)	(50)	(49)
Cardiovascular System				
Heart	(50)	(50)	(50)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Histiocytic sarcoma			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Hemangiosarcoma	1 (2%)			
Adrenal medulla	(50)	(50)	(49)	(49)
Pheochromocytoma malignant				1 (2%)
Pituitary gland	(47)	(50)	(48)	(46)
Histiocytic sarcoma		1 (2%)		
Pars distalis, adenoma	4 (9%)	7 (14%)	4 (8%)	6 (13%)
Thyroid gland	(45)	(48)	(48)	(48)
Follicular cell, adenoma	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Follicular cell, carcinoma				2 (4%)
General Body System				
None				

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Genital System				
Ovary	(45)	(50)	(49)	(49)
Cystadenoma	2 (4%)		2 (4%)	
Hemangiosarcoma		1 (2%)		
Uterus	(50)	(50)	(50)	(49)
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma	2 (4%)			
Leiomyoma			1 (2%)	
Polyp stromal				1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Histiocytic sarcoma			1 (2%)	
Lymph node	(5)	(2)	(4)	(4)
Lumbar, histiocytic sarcoma	1 (20%)			
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung				1 (25%)
Lymph node, mandibular	(48)	(46)	(48)	(49)
Histiocytic sarcoma			1 (2%)	
Lymph node, mesenteric	(48)	(50)	(49)	(48)
Histiocytic sarcoma			1 (2%)	
Spleen	(45)	(49)	(48)	(48)
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma			2 (4%)	
Thymus	(44)	(47)	(46)	(44)
Integumentary System				
Mammary gland	(48)	(48)	(48)	(49)
Carcinoma	1 (2%)			
Skin	(50)	(50)	(50)	(49)
Squamous cell carcinoma		1 (2%)		
Subcutaneous tissue, fibrosarcoma	2 (4%)	2 (4%)	1 (2%)	
Subcutaneous tissue, hemangioma			1 (2%)	
Subcutaneous tissue, sarcoma		1 (2%)	1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(49)
Osteosarcoma				1 (2%)
Skeletal muscle	(3)	(2)	(2)	(1)
Fibrosarcoma, metastatic, skin	1 (33%)	1 (50%)	1 (50%)	
Rhabdomyosarcoma				1 (100%)
Sarcoma, metastatic, skin		1 (50%)	1 (50%)	
Nervous System				
Brain	(50)	(50)	(50)	(49)
Histiocytic sarcoma		1 (2%)		1 (2%)
Spinal cord	(2)		(1)	

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Respiratory System				
Lung	(50)	(50)	(50)	(49)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Alveolar/bronchiolar carcinoma	3 (6%)			3 (6%)
Carcinoma, metastatic, harderian gland		1 (2%)		
Fibrosarcoma, metastatic, skin		1 (2%)		
Hemangiosarcoma		1 (2%)		
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Histiocytic sarcoma			2 (4%)	
Special Senses System				
Harderian gland	(2)	(4)	(4)	(1)
Adenoma	2 (100%)	3 (75%)	4 (100%)	1 (100%)
Carcinoma		1 (25%)		
Urinary System				
Kidney	(49)	(50)	(49)	(49)
Histiocytic sarcoma			2 (4%)	
Urinary bladder	(44)	(48)	(46)	(48)
Hemangioma		1 (2%)		
Histiocytic sarcoma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(49)
Histiocytic sarcoma	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Lymphoma malignant	14 (28%)	8 (16%)	8 (16%)	10 (20%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	28	38	41	44
Total primary neoplasms	45	63	63	82
Total animals with benign neoplasms	13	31	32	40
Total benign neoplasms	19	41	43	54
Total animals with malignant neoplasms	21	17	18	25
Total malignant neoplasms	26	22	20	28
Total animals with metastatic neoplasms	1	4	3	1
Total metastatic neoplasms	1	4	3	2

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

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

Number of Days on Study	242	519	554	554	569	613	617	627	652	690	701	715	717	717	717	730	730	730	730	730	731	731	731	731	731
Carcass ID Number	230	229	207	214	218	225	235	211	234	201	224	209	212	240	248	208	222	228	237	247	203	204	206	213	219
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma												X													
Hepatocellular adenoma		X										X													
Hepatocellular adenoma, multiple																									
Histiocytic sarcoma			X					X																	
Ito cell tumor malignant														X											
Mesentery							+					+			+										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																					X				
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth					+																				
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma														X											
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	M	M	M	M	M
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma															X				X		X				
Thyroid gland	+	A	+	+	+	A	M	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
Peritoneum													+												
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	A	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X					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 Anthraquinone: 0 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	221	226	227	233	243	244	245	246	250	202	205	210	215	216	217	220	223	231	232	236	238	239	241	242	249	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma			X																							2
Hepatocellular adenoma			X								X				X											5
Hepatocellular adenoma, multiple												X														1
Histiocytic sarcoma																										2
Ito cell tumor malignant																										1
Mesentery	+			+						+																6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma		X									X															3
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	39
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	47
Pars distalis, adenoma																								X		4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Follicular cell, adenoma												X														1
General Body System																										
Peritoneum																										1
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Cystadenoma															X						X					2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2

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

Number of Days on Study	242	519	554	554	569	613	617	627	652	690	701	715	717	717	717	730	730	730	730	730	731	731	731	731	731
Carcass ID Number	230	229	207	214	218	225	235	211	234	201	224	209	212	240	248	208	222	228	237	247	203	204	206	213	219
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node							A	+	+			+													
Lumbar, histiocytic sarcoma								X																	
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	A	+	+	+	A	A	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	M	+	A	+	+	+	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma														X											
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma							X							X											
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle										+				+											
Fibrosarcoma, metastatic, skin														X											
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve				+				+																	
Spinal cord				+				+																	
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Alveolar/bronchiolar carcinoma						X																		X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye			+						+																
Harderian gland																	+								
Adenoma																	X								
Urinary System																									
Kidney	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	A	+	+	+	A	A	+	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X					X																	
Lymphoma malignant		X							X			X								X	X		X	X	

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

Number of Days on Study	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	221	226	227	233	243	244	245	246	250	202	205	210	215	216	217	220	223	231	232	236	238	239	241	242	249	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node				+	+																					5
Lumbar, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Thymus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	48
Carcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle										+																3
Fibrosarcoma, metastatic, skin																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										2
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Alveolar/bronchiolar carcinoma													X													3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										2
Harderian gland																					+					2
Adenoma																					X					2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Histiocytic sarcoma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymphoma malignant					X		X		X		X			X	X										X	14

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 833 ppm

Number of Days on Study	499	519	549	575	611	648	694	703	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	285	259	261	290	294	284	273	267	255	257	277	288	297	300	252	253	254	263	265	266	268	269	270	276	283
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Hepatocellular carcinoma														X											
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma					X							X					X						X	X	
Hepatocellular adenoma, multiple									X		X					X				X	X	X			
Histiocytic sarcoma			X																						
Mesentery				+																					
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	+	+	+	+	M	+	M	+	+	+	+	+	M	+	+	+	M	M	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Pars distalis, adenoma								X	X	X		X													
Thyroid gland	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma														X											
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Vagina					+																				

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 833 ppm

Number of Days on Study	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	286	287	292	293	295	298	299	251	256	258	260	262	264	271	272	274	275	278	279	280	281	282	289	291	296	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
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																										1
Hepatocellular carcinoma, multiple				X																					X	2
Hepatocellular adenoma						X	X	X		X							X			X						11
Hepatocellular adenoma, multiple	X	X			X						X		X	X	X			X	X				X		X	17
Histiocytic sarcoma																										1
Mesentery		+			+														+							4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	M	+	+	+	+	+	+	M	M	+	+	+	M	+	M	+	+	M	+	+	+	+	M	+	36
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Pars distalis, adenoma											X						X		X							7
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell, adenoma																										1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Vagina																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 833 ppm

Number of Days on Study	499	519	549	575	611	648	694	703	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731
Carcass ID Number	285	259	261	290	294	284	273	267	255	257	277	288	297	300	252	253	254	263	265	266	268	269	270	276	283
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+											+													
Lymph node, mandibular	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																										
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																							X		
Subcutaneous tissue, fibrosarcoma										X															
Subcutaneous tissue, sarcoma						X																			
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle						+																			
Fibrosarcoma, metastatic, skin																									
Sarcoma, metastatic, skin						X																			
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								X	
Carcinoma, metastatic, harderian gland									X																
Fibrosarcoma, metastatic, skin										X															
Hemangiosarcoma					X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Eye			+																						
Harderian gland									+															+	
Adenoma																								X	
Carcinoma									X																
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																						X			
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Lymphoma malignant	X			X					X								X				X				

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 833 ppm

Number of Days on Study	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	286	287	292	293	295	298	299	251	256	258	260	262	264	271	272	274	275	278	279	280	281	282	289	291	296	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																										2
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
Subcutaneous tissue, fibrosarcoma											X															2
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle											+															2
Fibrosarcoma, metastatic, skin											X															1
Sarcoma, metastatic, skin																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Carcinoma, metastatic, harderian gland																										1
Fibrosarcoma, metastatic, skin																										1
Hemangiosarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye												+		+												3
Harderian gland					+															+						4
Adenoma					X															X						3
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangioma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant			X			X																		X		8

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 2,500 ppm

Number of Days on Study	491	505	568	589	599	624	626	627	631	675	675	676	700	700	711	730	730	730	730	730	730	730	730	730	730
Carcass ID Number	333	347	320	314	326	350	321	311	329	307	308	310	341	348	328	303	306	315	317	322	324	330	335	336	338
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma			X			X						X		X									X		X
Hepatocellular carcinoma, multiple																					X				
Hepatocellular adenoma							X		X			X		X			X				X				
Hepatocellular adenoma, multiple																				X		X	X		
Histiocytic sarcoma	X														X										
Mesentery				+				+						+	+										
Histiocytic sarcoma															X										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma															X										
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	M	M	+	M	+	+	+	M	M	+	M	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X																		
Thyroid gland	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																	X								
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 2,500 ppm

Number of Days on Study	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	
Carcass ID Number	344	304	312	313	316	318	319	327	331	337	339	340	342	343	345	346	349	301	302	305	309	323	325	332	334	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma							X																			7
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma							X			X				X	X			X	X	X	X					14
Hepatocellular adenoma, multiple		X	X		X	X		X	X		X					X	X							X		13
Histiocytic sarcoma																										2
Mesentery	+				+																					6
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	M	M	+	38
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma									X				X						X							4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell, adenoma																							X			2
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cystadenoma										X												X				2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma						X																				1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 2,500 ppm

Number of Days on Study	491	505	568	589	599	624	626	627	631	675	675	676	700	700	711	730	730	730	730	730	730	730	730	730	730
Carcass ID Number	3343	3347	3420	3414	3426	3520	3521	3411	3429	3407	3408	3410	3441	3448	3428	3403	3406	3415	3417	3422	3424	3430	3435	3436	3438
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Lymph node		+													+								+		
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Lymph node, mesenteric	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X																								
Spleen	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X														X										
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma								X																	
Subcutaneous tissue, hemangioma																								X	
Subcutaneous tissue, sarcoma																	X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle								+									+								
Fibrosarcoma, metastatic, skin								X																	
Sarcoma, metastatic, skin																	X								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve								+																	
Spinal cord								+																	
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																				X			X		
Hepatocellular carcinoma, metastatic, liver														X											
Histiocytic sarcoma	X														X										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																							+		
Harderian gland											+														
Adenoma											X														
Urinary System																									
Kidney	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X														X										
Urinary bladder	+	+	A	A	+	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma	X														X										
Lymphoma malignant		X						X					X										X		

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 2,500 ppm

Number of Days on Study	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	
Carcass ID Number	344	304	312	313	316	318	319	327	331	337	339	340	342	343	345	346	349	301	302	305	309	323	325	332	334	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node										+																4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										2
Thymus	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	48
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, hemangioma																										1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Fibrosarcoma, metastatic, skin																										1
Sarcoma, metastatic, skin																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										2
Hepatocellular carcinoma, metastatic, liver																										1
Histiocytic sarcoma																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										1
Harderian gland			+													+		+								4
Adenoma			X													X		X								4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymphoma malignant	X									X						X				X						8

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 7,500 ppm

Number of Days on Study	172	186	477	549	625	681	711	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731
Carcass ID Number	389	358	355	352	380	374	382	351	356	359	361	367	370	372	381	383	384	400	357	362	364	368	371	376
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma																								
Hepatocellular carcinoma							X	X							X									
Hepatocellular carcinoma, multiple																X			X					
Hepatocellular adenoma				X				X			X						X			X			X	
Hepatocellular adenoma, multiple						X			X	X		X		X	X	X		X	X			X		
Mesentery			+		+	+														+	+			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																								
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																								
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant				X																				
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X													X							
Thyroid gland	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																								
Follicular cell, carcinoma																								
General Body System																								
None																								
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																				X				

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 7,500 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	387	390	393	395	396	397	398	399	353	354	360	363	365	369	373	375	377	378	379	385	386	388	391	392	394	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatoblastoma																			X							1
Hepatocellular carcinoma																	X	X	X							6
Hepatocellular carcinoma, multiple																										2
Hepatocellular adenoma		X						X				X									X					10
Hepatocellular adenoma, multiple	X			X	X	X	X		X	X	X		X	X	X	X	X	X	X	X		X	X	X	X	30
Mesentery					+														+							7
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma																								X		1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar carcinoma, metastatic, lung										X																1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	+	+	+	+	+	+	M	+	M	+	+	M	+	+	+	+	M	+	+	M	+	+	M	38
Pituitary gland	+	+	+	M	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	46
Pars distalis, adenoma									X	X														X	X	6
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell, adenoma																						X		X		2
Follicular cell, carcinoma									X																X	2
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp stromal																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 7,500 ppm

Number of Days on Study	172	186	477	549	625	681	711	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731
Carcass ID Number	389	358	355	352	380	374	382	351	356	359	361	367	370	372	381	383	384	400	357	362	364	368	371	376	
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node					+		A																		
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mesenteric	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spleen	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Thymus	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Osteosarcoma		X																							
Skeletal muscle												+													
Rhabdomyosarcoma												X													
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Histiocytic sarcoma																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma									X																
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Special Senses System																									
Eye																									
Harderian gland																									
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Urinary bladder	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Histiocytic sarcoma																									
Lymphoma malignant					X				X				X	X	X										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Anthraquinone: 7,500 ppm

Number of Days on Study	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	387	390	393	395	396	397	398	399	353	354	360	363	365	369	373	375	377	378	379	385	386	388	391	392	394	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node				+		+				+																4
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung										X																1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	M	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Osteosarcoma																										1
Skeletal muscle																										1
Rhabdomyosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma															X											1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma															X									X		3
Alveolar/bronchiolar carcinoma	X									X									X							3
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye						+																				1
Harderian gland														+												1
Adenoma														X												1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma															X											1
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 Anthraquinone

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Harderian Gland: Adenoma				
Overall rate[a]	2/50 (4%)	3/50 (6%)	4/50 (8%)	1/50 (2%)
Adjusted rate[b]	4.5%	6.4%	8.9%	2.2%
Terminal rate[c]	2/35 (6%)	3/42 (7%)	3/35 (9%)	1/42 (2%)
First incidence (days)	730 (T)	730 (T)	675	730 (T)
Poly-3 test[d]	P=0.299N	P=0.518	P=0.339	P=0.491N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	4/50 (8%)	1/50 (2%)
Adjusted rate	4.5%	8.6%	8.9%	2.2%
Terminal rate	2/35 (6%)	4/42 (10%)	3/35 (9%)	1/42 (2%)
First incidence (days)	730 (T)	730 (T)	675	730 (T)
Poly-3 test	P=0.238N	P=0.356	P=0.339	P=0.491N
Liver: Hepatocellular Adenoma				
Overall rate	6/49 (12%)	28/50 (56%)	27/50 (54%)	40/49 (82%)
Adjusted rate	13.4%	59.5%	58.9%	87.2%
Terminal rate	4/35 (11%)	27/42 (64%)	23/35 (66%)	38/42 (91%)
First incidence (days)	519	611	626	549
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rate	2/49 (4%)	3/50 (6%)	8/50 (16%)	8/49 (16%)
Adjusted rate	4.5%	6.4%	17.4%	17.7%
Terminal rate	1/35 (3%)	3/42 (7%)	4/35 (11%)	7/42 (17%)
First incidence (days)	715	730 (T)	568	711
Poly-3 test	P=0.031	P=0.524	P=0.051	P=0.048
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	6/49 (12%)	30/50 (60%)	30/50 (60%)	41/49 (84%)
Adjusted rate	13.4%	63.8%	64.2%	89.3%
Terminal rate	4/35 (11%)	29/42 (69%)	24/35 (69%)	38/42 (91%)
First incidence (days)	519	611	568	549
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	2/49 (4%)	3/50 (6%)	8/50 (16%)	8/49 (16%)
Adjusted rate	4.5%	6.4%	17.4%	17.7%
Terminal rate	1/35 (3%)	3/42 (7%)	4/35 (11%)	7/42 (17%)
First incidence (days)	715	730 (T)	568	711
Poly-3 test	P=0.031	P=0.524	P=0.051	P=0.048
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	6/49 (12%)	30/50 (60%)	30/50 (60%)	41/49 (84%)
Adjusted rate	13.4%	63.8%	64.2%	89.3%
Terminal rate	4/35 (11%)	29/42 (69%)	24/35 (69%)	38/42 (91%)
First incidence (days)	519	611	568	549
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	3/49 (6%)
Adjusted rate	2.2%	2.1%	4.5%	6.7%
Terminal rate	1/35 (3%)	1/42 (2%)	2/35 (6%)	3/42 (7%)
First incidence (days)	730 (T)	730 (T)	730 (T)	730 (T)
Poly-3 test	P=0.179	P=0.752N	P=0.500	P=0.308
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	3/49 (6%)
Adjusted rate	6.6%	0.0%	0.0%	6.7%
Terminal rate	2/35 (6%)	0/42 (0%)	0/35 (0%)	3/42 (7%)
First incidence (days)	613	—[e]	—	730 (T)
Poly-3 test	P=0.284	P=0.113N	P=0.120N	P=0.661
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	2/50 (4%)	6/49 (12%)
Adjusted rate	8.8%	2.1%	4.5%	13.3%
Terminal rate	3/35 (9%)	1/42 (2%)	2/35 (6%)	6/42 (14%)
First incidence (days)	613	730 (T)	730 (T)	730 (T)
Poly-3 test	P=0.089	P=0.169N	P=0.341N	P=0.368
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/47 (9%)	7/50 (14%)	4/48 (8%)	6/46 (13%)
Adjusted rate	9.4%	15.0%	9.2%	14.1%
Terminal rate	3/33 (9%)	6/42 (14%)	3/35 (9%)	5/39 (13%)
First incidence (days)	717	703	626	549
Poly-3 test	P=0.428	P=0.318	P=0.632N	P=0.372
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	4.4%	6.4%	4.4%	0.0%
Terminal rate	0/35 (0%)	2/42 (5%)	1/35 (3%)	0/42 (0%)
First incidence (days)	617	648	627	—
Poly-3 test	P=0.117N	P=0.517	P=0.693	P=0.234N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.7%	0.0%	0.0%	2.2%
Terminal rate	3/35 (9%)	0/42 (0%)	0/35 (0%)	1/42 (2%)
First incidence (days)	730 (T)	—	—	730 (T)
Poly-3 test	P=0.450N	P=0.112N	P=0.119N	P=0.297N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/45 (2%)	1/48 (2%)	2/48 (4%)	4/48 (8%)
Adjusted rate	2.4%	2.2%	4.6%	9.1%
Terminal rate	1/35 (3%)	1/42 (2%)	2/35 (6%)	4/42 (10%)
First incidence (days)	730 (T)	730 (T)	730 (T)	730 (T)
Poly-3 test	P=0.078	P=0.741N	P=0.519	P=0.198

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

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
All Organs: Malignant Lymphoma				
Overall rate	14/50 (28%)	8/50 (16%)	8/50 (16%)	10/50 (20%)
Adjusted rate	30.6%	16.7%	17.4%	21.6%
Terminal rate	11/35 (31%)	6/42 (14%)	5/35 (14%)	9/42 (21%)
First incidence (days)	519	499	505	625
Poly-3 test	P=0.413N	P=0.089N	P=0.107N	P=0.229N
All Organs: Benign Neoplasms				
Overall rate	13/50 (26%)	31/50 (62%)	32/50 (64%)	40/50 (80%)
Adjusted rate	28.5%	65.7%	69.5%	85.7%
Terminal rate	10/35 (29%)	29/42 (69%)	27/35 (77%)	38/42 (91%)
First incidence (days)	519	611	626	549
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
All Organs: Malignant Neoplasms				
Overall rate	21/50 (42%)	17/50 (34%)	18/50 (36%)	25/50 (50%)
Adjusted rate	44.1%	34.6%	37.6%	52.2%
Terminal rate	13/35 (37%)	12/42 (29%)	9/35 (26%)	21/42 (50%)
First incidence (days)	519	499	491	186
Poly-3 test	P=0.099	P=0.227N	P=0.327N	P=0.281
All Organs: Benign or Malignant Neoplasms				
Overall rate	28/50 (56%)	38/50 (76%)	41/50 (82%)	44/50 (88%)
Adjusted rate	58.8%	77.2%	83.9%	91.5%
Terminal rate	19/35 (54%)	32/42 (76%)	29/35 (83%)	39/42 (93%)
First incidence (days)	519	499	491	186
Poly-3 test	P<0.001	P=0.039	P=0.004	P<0.001

(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 is the P value associated with the trend test. Beneath the exposed group incidences 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 D4a
Historical Incidence of Liver Neoplasms in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	17/51	4/51	20/51
Manganese (II) sulfate monohydrate	12/51	3/51	13/51
Oxazepam	25/50	9/50	28/50
Primadone	15/50	3/50	16/50
Triamterene	10/50	4/50	13/50
Triamterene	7/50	5/50	10/50
Tricresyl phosphate	12/50	10/50	21/50
Overall Historical Incidence			
Total (%)	203/852 (23.8%)	98/852 (11.5%)	273/852 (32.0%)
Mean ± standard deviation	23.8% ± 10.0%	11.5% ± 4.5%	32.0% ± 9.6%
Range	12%-50%	6%-20%	18%-56%
	Hepatoblastoma	**Hepatocellular Carcinoma or Hepatoblastoma**	**Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma**
Historical Incidence at Battelle Columbus Laboratories			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	0/51	4/51	20/51
Manganese (II) sulfate monohydrate	0/51	3/51	13/51
Oxazepam	0/50	9/50	28/50
Primadone	1/50	4/50	16/50
Triamterene	0/50	4/50	13/50
Triamterene	0/50	5/50	10/50
Tricresyl phosphate	0/50	10/50	21/50
Overall Historical Incidence			
Total (%)	2/852 (0.2%)	100/852 (11.7%)	273/852 (32.0%)
Mean ± standard deviation	0.2% ± 0.7%	11.7% ± 4.4%	32.0% ± 9.6%
Range	0%-2%	6%-20%	18%-56%

[a] Data as of November 3, 1998

TABLE D4b
Historical Incidence of Thyroid Gland Follicular Cell Neoplasms in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratory			
4,4-Thiobis-(6-*t*-butyl-*m*-cresol)	0/51	0/51	0/51
Manganese (II) sulfate monohydrate	2/50	0/50	2/50
Oxazepam	0/50	0/50	0/50
Pentachlorophenol	1/50	0/50	1/50
Primadone	1/49	1/49	2/49
Triamterene	0/50	0/50	0/50
Tricresyl phosphate	1/49	0/49	1/49
Overall Historical Incidence			
Total (%)	13/847 (1.5%)	2/847 (0.2%)	15/847 (1.8%)
Mean ± standard deviation	1.5% ± 1.6%	0.2% ± 0.7%	1.8% ± 1.7%
Range	0%-6%	0%-2%	0%-6%

[a] Data as of November 3, 1998

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Anthraquinone[a]

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths			3	
Moribund	6	3	4	2
Natural deaths	9	5	8	5
Survivors				
Terminal sacrifice	35	42	35	42
Missing				1
Animals examined microscopically	50	50	50	49
Alimentary System				
Esophagus	(50)	(49)	(50)	(49)
Inflammation, chronic	1 (2%)			
Intestine small, duodenum	(50)	(50)	(50)	(48)
Ulcer	1 (2%)		1 (2%)	
Liver	(49)	(50)	(50)	(49)
Basophilic focus	1 (2%)	1 (2%)		3 (6%)
Clear cell focus	4 (8%)	1 (2%)	1 (2%)	3 (6%)
Degeneration, diffuse, fatty	1 (2%)	1 (2%)		
Degeneration, fatty, focal	2 (4%)	3 (6%)	1 (2%)	9 (18%)
Eosinophilic focus	6 (12%)	15 (30%)	11 (22%)	22 (45%)
Fatty change, focal	5 (10%)	2 (4%)	1 (2%)	3 (6%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Infiltration cellular, lymphocyte				3 (6%)
Mineralization, focal				1 (2%)
Mixed cell focus	4 (8%)	2 (4%)	2 (4%)	2 (4%)
Necrosis, focal	5 (10%)	3 (6%)	1 (2%)	1 (2%)
Tension lipidosis	1 (2%)			
Bile duct, cyst	3 (6%)	2 (4%)	2 (4%)	
Bile duct, hyperplasia			1 (2%)	
Centrilobular, atrophy	1 (2%)			
Centrilobular, degeneration, fatty	1 (2%)			1 (2%)
Centrilobular, hypertrophy	1 (2%)	27 (54%)	22 (44%)	39 (80%)
Hepatocyte, erythrophagocytosis	1 (2%)			
Serosa, inflammation, chronic	1 (2%)			
Mesentery	(6)	(4)	(6)	(7)
Inflammation, chronic	1 (17%)			
Inflammation, chronic active			1 (17%)	
Artery, thrombosis	1 (17%)			
Fat, necrosis	4 (67%)	3 (75%)	2 (33%)	7 (100%)
Lymphatic, angiectasis			1 (17%)	
Pancreas	(50)	(50)	(50)	(49)
Hyperplasia, focal		2 (4%)		
Inflammation, chronic	1 (2%)			
Acinus, atrophy	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Duct, cyst			1 (2%)	1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(49)
Inflammation, suppurative				1 (2%)
Ulcer	4 (8%)	1 (2%)	1 (2%)	2 (4%)
Epithelium, hyperplasia, focal		1 (2%)	1 (2%)	1 (2%)
Serosa, inflammation, chronic	1 (2%)			

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

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Anthraquinone

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Alimentary System (continued)				
Stomach, glandular	(50)	(50)	(50)	(49)
Ulcer	1 (2%)		1 (2%)	
Epithelium, hyperplasia		2 (4%)		
Epithelium, hyperplasia, focal				1 (2%)
Serosa, inflammation, chronic	1 (2%)			
Tooth	(1)			
Peridontal tissue, inflammation, granulomatous	1 (100%)			
Cardiovascular System				
Blood vessel	(50)	(50)	(49)	(49)
Aorta, mineralization	2 (4%)		1 (2%)	
Heart	(50)	(50)	(50)	(49)
Thrombosis		1 (2%)		
Myocardium, degeneration	2 (4%)		1 (2%)	1 (2%)
Myocardium, mineralization	2 (4%)			
Valve, inflammation		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Hyperplasia, focal		1 (2%)	1 (2%)	2 (4%)
Necrosis		1 (2%)		
Adrenal medulla	(50)	(50)	(49)	(49)
Hyperplasia, focal				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(49)
Hyperplasia	6 (12%)	13 (26%)	10 (20%)	14 (29%)
Pituitary gland	(47)	(50)	(48)	(46)
Angiectasis	2 (4%)		1 (2%)	
Atrophy		1 (2%)		
Cyst				1 (2%)
Pars distalis, hyperplasia, focal	8 (17%)	4 (8%)	12 (25%)	6 (13%)
Thyroid gland	(45)	(48)	(48)	(48)
Inflammation, acute, focal	1 (2%)			
Follicle, cyst	2 (4%)	1 (2%)		
Follicular cell, hyperplasia	10 (22%)	14 (29%)	16 (33%)	15 (31%)
General Body System				
Peritoneum	(1)			
Necrosis	1 (100%)			
Genital System				
Ovary	(45)	(50)	(49)	(49)
Angiectasis				2 (4%)
Cyst	3 (7%)	4 (8%)	7 (14%)	5 (10%)
Granulosa cell, hyperplasia	1 (2%)			
Uterus	(50)	(50)	(50)	(49)
Angiectasis	2 (4%)		1 (2%)	1 (2%)
Inflammation, chronic	1 (2%)			
Inflammation, suppurative	1 (2%)		1 (2%)	
Thrombosis			1 (2%)	
Endometrium, hyperplasia, cystic	35 (70%)	38 (76%)	23 (46%)	39 (80%)
Vagina		(1)		
Hemorrhage		1 (100%)		

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Anthraquinone

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Myeloid cell, hyperplasia				1 (2%)
Lymph node	(5)	(2)	(4)	(4)
Lumbar, ectasia		1 (50%)		
Lumbar, hemorrhage			1 (25%)	
Mediastinal, inflammation, chronic	1 (20%)			
Lymph node, mandibular	(48)	(46)	(48)	(49)
Hyperplasia, lymphoid		1 (2%)		
Inflammation, suppurative	1 (2%)			
Lymph node, mesenteric	(48)	(50)	(49)	(48)
Ectasia				1 (2%)
Spleen	(45)	(49)	(48)	(48)
Hematopoietic cell proliferation	9 (20%)	17 (35%)	17 (35%)	26 (54%)
Pigmentation	1 (2%)			
Capsule, inflammation, chronic	1 (2%)			
Lymphoid follicle, hyperplasia	8 (18%)	4 (8%)	5 (10%)	6 (13%)
Thymus	(44)	(47)	(46)	(44)
Hyperplasia, lymphoid	1 (2%)			
Integumentary System				
Mammary gland	(48)	(48)	(48)	(49)
Inflammation, suppurative	1 (2%)			
Skin	(50)	(50)	(50)	(49)
Hair follicle, atrophy, focal			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(49)
Fibrosis	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Skeletal muscle	(3)	(2)	(2)	(1)
Hemorrhage, acute	1 (33%)			
Inflammation, chronic	1 (33%)			
Nervous System				
Brain	(50)	(50)	(50)	(49)
Hemorrhage				1 (2%)
Hydrocephalus			1 (2%)	
Hypothalamus, compression		1 (2%)	1 (2%)	1 (2%)
Hypothalamus, degeneration	1 (2%)			
Hypothalamus, necrosis		1 (2%)		
Peripheral nerve	(2)		(1)	
Axon, degeneration	2 (100%)			
Spinal cord	(2)		(1)	
Axon, nerve, degeneration	1 (50%)			
Respiratory System				
Lung	(50)	(50)	(50)	(49)
Congestion			2 (4%)	
Hematopoietic cell proliferation				1 (2%)
Hemorrhage		2 (4%)	1 (2%)	
Hemorrhage, chronic, focal	1 (2%)			
Inflammation, chronic			1 (2%)	

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Anthraquinone

	0 ppm	833 ppm	2,500 ppm	7,500 ppm
Respiratory System (continued)				
Lung (continued)	(50)	(50)	(50)	(49)
Alveolar epithelium, hyperplasia, focal			2 (4%)	
Bronchus, foreign body	1 (2%)			
Interstitium, inflammation, chronic, focal				1 (2%)
Interstitium, mineralization	1 (2%)			
Perivascular, edema			1 (2%)	
Nose	(50)	(50)	(49)	(49)
Inflammation, suppurative	2 (4%)			
Special Senses System				
Eye	(2)	(3)	(1)	(1)
Degeneration	1 (50%)	1 (33%)	1 (100%)	1 (100%)
Cornea, inflammation, chronic active		1 (33%)		
Lens, cataract	1 (50%)			
Urinary System				
Kidney	(49)	(50)	(49)	(49)
Glomerulosclerosis	1 (2%)			
Infarct		2 (4%)		
Inflammation, suppurative	1 (2%)	1 (2%)		
Nephropathy	4 (8%)	3 (6%)	3 (6%)	7 (14%)
Pigmentation	1 (2%)			
Pelvis, inflammation, chronic active	1 (2%)			
Renal tubule, cyst				1 (2%)
Renal tubule, hyperplasia, focal			1 (2%)	1 (2%)
Urinary bladder	(44)	(48)	(46)	(48)
Edema				1 (2%)
Transitional epithelium, inclusion body, intracytoplasmic		40 (83%)	43 (93%)	46 (96%)

APPENDIX E
GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL E-2
MOUSE BONE MARROW MICRONUCLEUS TEST PROTOCOL E-2
MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL E-3
EVALUATION PROTOCOL E-3
RESULTS E-3
TABLE E1 Mutagenicity of Anthraquinone (97% Pure) in *Salmonella typhimurium* E-5
TABLE E2 Mutagenicity of Anthraquinone (100% Pure) in *Salmonella typhimurium* E-6
TABLE E3 Mutagenicity of 1-Hydroxyanthraquinone in *Salmonella typhimurium* E-7
TABLE E4 Mutagenicity of 2-Hydroxyanthraquinone in *Salmonella typhimurium* E-8
TABLE E5 Mutagenicity of 1-Nitroanthracene in *Salmonella typhimurium* E-10
TABLE E6 Mutagenicity of 2-Nitroanthracene in *Salmonella typhimurium* E-11
TABLE E7 Mutagenicity of 9-Nitroanthracene in *Salmonella typhimurium* E-12
TABLE E8 Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with Anthraquinone by Intraperitoneal Injection E-13
TABLE E9 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Anthraquinone (99.9% Pure) in Feed for 14 Weeks E-14

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1988) **or Zeiger *et al.* (1992). Anthraquinone (97% pure) was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). Anthraquinone (100% pure), 1- and 2-hydroxyanthraquinone, and 1-, 2-, and 9-nitroanthracene were sent to the study laboratory as coded aliquots from Battelle Columbus Operations (Columbus, OH).** The compounds were 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. Anthraquinone (100% pure) and 1-hydroxyanthraquinone were also incubated with strain TA102. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of anthraquinone. In the absence of toxicity, 10,000 μg/plate was selected as the high dose.

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

MOUSE BONE MARROW MICRONUCLEUS TEST PROTOCOL

The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male B6C3F$_1$ mice were injected intraperitoneally three times at 24-hour intervals with anthraquinone dissolved in corn oil. Solvent control animals were injected with corn oil only. The positive control animals received injections of 12.5 mg/kg dimethylbenzanthracene. The animals were killed 24 hours after the third injection, and 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.

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 dose group is less than or equal to 0.025 divided by the number of dose 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

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 $B6C3F_1$ mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) **and the percent of polychromatic erythrocytes (PCEs) in 1,000 total erythrocytes** in each of five animals per exposure group.

The results were tabulated and the frequency of micronucleated cells among NCEs was analyzed as described for PCEs in the bone marrow micronucleus test protocol.

EVALUATION PROTOCOL

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

RESULTS

Anthraquinone (97% pure) (33 to 2,500 μg/plate) was mutagenic in *S. typhimurium* strains TA98 and TA100, with and without hamster or rat liver S9 enzymes (Zeiger *et al.,* 1988; Table E1). Subsequent testing with a 100% pure sample of anthraquinone (100 to 10,000 μg/plate) showed no detectable mutagenic response in TA98, TA100, or TA102, with or without 10% rat liver S9 (Table E2). Additional testing of several substituted anthraquinones revealed an interesting pattern of responses. 1-Hydroxyanthraquinone (up to 10,000 μg/plate) was not mutagenic in TA98, TA100, or TA102, with or without rat liver S9 (Table E3). 2-Hydroxyanthraquinone (3.3 to 450 μg/plate) was mutagenic at low doses in TA98 in the absence of rat liver S9; it was not reproducibly mutagenic with S9, and no mutagenic response was seen with this compound in TA100, with or without S9 (Table E4). 1-, 2-, and 9-Nitroanthracene were all mutagenic in TA98 and TA100, with and without 10% S9 (Tables E5, E6, and E7); based on the magnitudes of the responses and the lowest effective concentrations required to produce a clear increase in mutant colonies, 2-nitroanthracene was the strongest mutagen of these three substituted anthracenes. 9-nitroanthracene was more strongly mutagenic with S9 than without S9; both trials conducted in the absence of S9 were positive, but the peak response was less than twice the control frequency. In contrast to the pattern of mutagenicity seen with 9-nitroanthracene, 1-nitroanthracene produced responses of similar magnitude with and without S9 while 2-nitroanthracene was clearly more mutagenic without S9.

Negative results were obtained in an acute bone marrow micronucleus test performed with male mice administered 500 to 2,000 mg/kg anthraquinone via intraperitoneal injection (Table E8). However, when male and female mice administered anthraquinone (99.9% pure) in feed (1,875 to 30,000 ppm) for 14 weeks were examined for frequency of micronucleated NCEs in the peripheral blood, significant increases over the control frequencies were noted in male and female mice at the highest exposure concentration (Table E9). Although only the 30,000 ppm female group differed significantly from the control frequency by pairwise comparison, both data sets yielded positive trend tests, and the peripheral blood micronucleus test was judged to be positive for both male and female mice. **Evidence of increased erythropoiesis in treated mice was demonstrated by the slightly elevated percent PCE values in several of the exposure groups, mostly in exposed female mice. The data do not demonstrate a direct correlation between percent PCEs and micronucleus frequency except in the high exposure concentration groups where both male and female mice showed the highest frequencies of micronucleated erythrocytes and the highest percent PCE values. An increased rate of erythropoiesis may have contributed to the micronucleus responses seen in the high exposure concentration groups, because increased cell proliferation can produce increased levels of mitotic errors.**

TABLE E1
Mutagenicity of Anthraquinone (97% Pure) 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
TA100	0	80 ± 0.9	79 ± 2.7	89 ± 9.3	88 ± 2.8	106 ± 11.6	102 ± 4.9
	33	98 ± 4.3	80 ± 2.0	101 ± 2.1	126 ± 5.9	117 ± 3.8	95 ± 2.9
	100	106 ± 5.7	102 ± 6.9	123 ± 2.6	127 ± 11.7	111 ± 1.5	126 ± 5.1
	333	135 ± 14.7	145 ± 6.3	136 ± 1.2	171 ± 9.3	120 ± 5.3	130 ± 2.5
	1,000	310 ± 10.9	296 ± 15.6	250 ± 4.7	246 ± 13.6	206 ± 8.6	184 ± 2.6
	2,500	602 ± 30.7	670 ± 23.5[d]	459 ± 19.7	466 ± 23.2[d]	391 ± 9.1[d]	341 ± 25.7[d]
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control[c]		421 ± 28.0	234 ± 14.4	398 ± 14.1	330 ± 14.2	292 ± 3.9	268 ± 5.7
TA98	0	15 ± 1.7	15 ± 2.7	20 ± 2.7	33 ± 3.7		
	33	43 ± 4.4	51 ± 6.1	28 ± 1.2	39 ± 4.8		
	100	70 ± 10.5	112 ± 1.2	34 ± 3.5	52 ± 1.5		
	333	225 ± 12.2	265 ± 5.7	58 ± 4.9	81 ± 3.8		
	1,000	723 ± 29.2	738 ± 8.7	170 ± 7.1	187 ± 22.4		
	2,500	1,497 ± 55.9	1,388 ± 29.4[d]	401 ± 8.7	492 ± 40.4[d]		
Trial summary		Positive	Positive	Positive	Positive		
Positive control		162 ± 9.9	157 ± 7.4	82 ± 5.5	79 ± 5.2		

		+ 30% rat S9 Trial 1	+ 30% rat S9 Trial 2	+ 30% rat S9 Trial 3
TA98 (continued)	0	17 ± 1.5	24 ± 2.2	27 ± 5.5
	33	25 ± 4.1	30 ± 4.7	39 ± 3.8
	100	31 ± 6.7	40 ± 4.1	43 ± 13.4
	333	43 ± 0.9	57 ± 4.5	73 ± 0.9
	1,000	122 ± 2.8	145 ± 12.7	157 ± 11.4
	2,500	371 ± 4.3	421 ± 28.6[d]	356 ± 29.6[d]
Trial summary		Positive	Positive	Positive
Positive control		33 ± 1.7	101 ± 3.7	82 ± 4.2

[a] Study was performed at Microbiological Associates. The detailed protocol and these data are presented by Zeiger *et al.* (1988). 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
Mutagenicity of Anthraquinone (100% Pure) in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9	Revertants/Plate[b] +10% rat S9
TA102	0	261 ± 12.0	360 ± 22.1
	100	264 ± 21.5	347 ± 11.3
	333	277 ± 6.4	327 ± 15.1
	1,000	312 ± 9.4[d]	291 ± 13.3[d]
	3,333	295 ± 14.7[d]	340 ± 6.6[d]
	10,000	331 ± 26.8[d]	335 ± 7.0[d]
Trial summary		Negative	Negative
Positive control[c]		1,196 ± 26.7	1,449 ± 19.8
TA100	0	120 ± 4.6	102 ± 2.5
	100	120 ± 3.5	111 ± 7.5
	333	122 ± 2.3	112 ± 7.2
	1,000	125 ± 12.3[d]	120 ± 7.8[d]
	3,333	108 ± 6.2[d]	111 ± 9.3[d]
	10,000	130 ± 7.2[d]	102 ± 15.7[d]
Trial summary		Negative	Negative
Positive control		639 ± 29.5	628 ± 12.7
TA98	0	12 ± 0.9	14 ± 1.5
	100	11 ± 0.9	15 ± 0.3
	333	13 ± 1.0[e]	13 ± 1.3
	1,000	13 ± 2.0[d]	20 ± 2.4[d]
	3,333	12 ± 0.3[d]	14 ± 1.2[d]
	10,000	12 ± 1.5[d]	15 ± 1.2[d]
Trial summary		Negative	Negative
Positive control		106 ± 4.7	282 ± 24.5

[a] Study was performed at BioReliance Corporation (Rockville, MD). 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 except where noted..
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and mitomycin-C (TA102). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d] Precipitate on plate
[e] Mean ± standard error from two plates; third plate contaminated.

TABLE E3
Mutagenicity of 1-Hydroxyanthraquinone in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9 Trial 1	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA102	0	302 ± 23.9	358 ± 29.8	214 ± 10.0
	100	356 ± 14.5	437 ± 30.7	208 ± 6.4
	333	375 ± 13.8	452 ± 12.4	205 ± 3.5
	1,000	380 ± 15.8[d]	498 ± 7.0[d]	168 ± 16.9[d]
	3,333	427 ± 29.7[d]	405 ± 28.6[d]	198 ± 9.4[d]
	10,000	434 ± 33.3[d]	526 ± 18.4[d]	209 ± 8.2[d]
Trial summary		Negative	Negative	Negative
Positive control[c]		1,259 ± 92.9	1,150 ± 30.8	1,793 ± 172.7
TA100	0	173 ± 16.8	233 ± 10.1	
	100	203 ± 9.7	254 ± 12.0	
	333	194 ± 8.0	245 ± 16.6	
	1,000	212 ± 10.4[d]	245 ± 15.5	
	3,333	228 ± 16.6[d]	242 ± 11.6	
	10,000	210 ± 6.2[d]	259 ± 4.0	
Trial summary		Negative	Negative	
Positive control		558 ± 30.8	1,390 ± 296.8	
TA98	0	17 ± 3.0	17 ± 3.5	
	100	14 ± 1.7	17 ± 1.9	
	333	11 ± 1.2	22 ± 1.0	
	1,000	15 ± 1.5[d]	24 ± 0.3[d]	
	3,333	12 ± 0.9[d]	27 ± 0.9[d]	
	10,000	13 ± 1.8[d]	25 ± 2.7[d]	
Trial summary		Negative	Negative	
Positive control		129 ± 6.5	555 ± 35.3	

[a] Study was performed at BioReliance Corporation (Rockville, MD). The detailed protocol is presented by Zeiger *et al.* (1992). 0 μg/plate was the solvent control (dimethylsulfoxide).
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and mitomycin-C (TA102). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d] Precipitate on plate

TABLE E4
Mutagenicity of 2-Hydroxyanthraquinone in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9 Trial 1	Trial 2	Trial 3	Trial 4
TA100	0.0	152 ± 8.3	114 ± 11.0	153 ± 0.6	208 ± 6.7
	3.3		95 ± 7.7		188 ± 2.7
	10	168 ± 12.1	100 ± 1.5	160 ± 28.7	200 ± 12.1
	33	153 ± 3.4	101 ± 3.9	245 ± 10.8	250 ± 22.9
	100	164 ± 19.9	108 ± 3.5	256 ± 5.2	247 ± 22.7
	200		96 ± 4.5	259 ± 16.3	
	333	Toxic[d]		167 ± 12.2[d]	156 ± 10.3[d]
	450	Toxic[d]			
Trial summary		Negative	Negative	Weakly positive	Negative
Positive control[c]		586 ± 23.6	544 ± 43.3	706 ± 41.6	591 ± 15.6
		+ 10% rat S9 Trial 1	Trial 2	Trial 3	Trial 4
TA100 (continued)	0.0	150 ± 8.4	127 ± 6.4	169 ± 2.7	220 ± 5.8
	3.3		131 ± 1.7		258 ± 14.5
	10	219 ± 24.2	115 ± 4.8	246 ± 5.2	288 ± 9.8
	33	172 ± 8.1	106 ± 2.7	174 ± 43.7	258 ± 2.9
	100	126 ± 10.3	96 ± 3.1	231 ± 11.4	245 ± 15.0
	200		102 ± 1.8	181 ± 6.9	
	333	Toxic		145 ± 13.5[d]	222 ± 9.2[d]
	450	Toxic[d]			
Trial summary		Equivocal	Negative	Equivocal	Negative
Positive control		567 ± 13.7	671 ± 16.2	1,481 ± 30.9	699 ± 47.5

TABLE E4
Mutagenicity of 2-Hydroxyanthraquinone in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/Plate -S9 Trial 1	Trial 2	Trial 3	Trial 4
TA98	0.0	11 ± 2.6	18 ± 1.8	16 ± 3.2	16 ± 3.2
	3.3				22 ± 2.7
	10	26 ± 1.2	25 ± 4.1	37 ± 7.4	47 ± 7.7
	33	47 ± 0.7	45 ± 5.2	107 ± 4.3	84 ± 7.9
	100	157 ± 9.1	246 ± 4.7	227 ± 6.0	329 ± 31.5
	200		160 ± 18.7	191 ± 6.5	
	333	77 ± 8.8[d]	169 ± 18.7	184 ± 3.5[d]	214 ± 5.7[d]
	450	53 ± 3.4[d]			
Trial summary		Positive	Positive	Positive	Positive
Positive control		104 ± 14.0	106 ± 10.9	145 ± 6.2	430 ± 7.5
		+ 10% rat S9 Trial 1	Trial 2	Trial 3	Trial 4
TA98 (continued)	0.0	14 ± 2.5	19 ± 2.3	23 ± 1.8	34 ± 2.8
	3.3				
	10	20 ± 0.7	18 ± 2.3	35 ± 6.7	38 ± 3.2
	33	27 ± 4.4	16 ± 0.3	39 ± 3.5	34 ± 2.1
	100	42 ± 0.9	17 ± 1.0	67 ± 3.2	55 ± 8.0
	200		27 ± 0.6		
	333	57 ± 4.3[d]	29 ± 4.3	101 ± 11.5[d]	116 ± 9.4[d]
	450	52 ± 5.8[e]		90 ± 2.0[d]	68 ± 9.9[d]
Trial summary		Positive	Negative	Positive	Positive
Positive control		276 ± 5.4	437 ± 39.3	669 ± 16.4	385 ± 28.7

[a] Study was performed at BioReliance Corporation (Rockville, MD). The detailed protocol is presented by Zeiger *et al*. (1992). 0 μg/plate was the solvent control (dimethylsulfoxide).
[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
[e] Background obscured by precipitate

TABLE E5
Mutagenicity of 1-Nitroanthracene in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9 Trial 1	-S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0.00	147 ± 20.7	152 ± 10.8	173 ± 6.6	134 ± 6.1
	0.10	175 ± 14.5	210 ± 6.2		
	0.33	189 ± 0.9	234 ± 23.8	159 ± 5.3	
	1.0	261 ± 41.1	325 ± 15.6	155 ± 8.5	152 ± 5.6
	3.3	609 ± 20.9	652 ± 49.9	209 ± 7.0	178 ± 6.4
	10	1,006 ± 69.8	713 ± 112.1	363 ± 9.2	291 ± 12.9
	20				520 ± 65.0
	33			744 ± 21.2	636 ± 30.3
Trial summary		Positive	Positive	Positive	Positive
Positive control[c]		635 ± 12.3	631 ± 4.0	650 ± 21.5	646 ± 17.9
TA98	0.00	17 ± 3.5	11 ± 0.0	22 ± 1.9	16 ± 3.2
	0.10	28 ± 3.1	20 ± 1.5		
	0.33	65 ± 14.3	33 ± 1.5	23 ± 2.9	
	1.0	157 ± 13.9	102 ± 6.2	23 ± 4.3	62 ± 15.2
	3.3	433 ± 25.9	296 ± 33.8	29 ± 2.6	55 ± 6.0
	10	581 ± 34.3	723 ± 66.9	92 ± 13.5	104 ± 16.2[d]
	20				151 ± 75.6[d]
	33			651 ± 17.4	0 ± 0.0[d]
Trial summary		Positive	Positive	Positive	Positive
Positive control		101 ± 7.4	116 ± 5.8	250 ± 19.2	96 ± 5.8

[a] Study was performed at BioReliance Corporation (Rockville, MD). The detailed protocol is presented by Zeiger *et al.* (1992). 0 μg/plate was the solvent control (acetone).
[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] Slight toxicity

TABLE E6
Mutagenicity of 2-Nitroanthracene in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9 Trial 1	-S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0.000	163 ± 6.9	118 ± 7.9	126 ± 6.6	122 ± 4.8
	0.033		517 ± 33.4	340 ± 28.7	132 ± 2.9
	0.10		1,228 ± 47.5	203 ± 19.8	250 ± 27.9
	0.33	2,084 ± 110.4	2,040 ± 27.3	594 ± 202.2	376 ± 19.5
	1.0	1,720 ± 147.2	1,710 ± 82.2	825 ± 67.4	735 ± 5.7
	3.3	0 ± 0.0[d]	0 ± 0.0[d]	1,385 ± 9.6	1,404 ± 34.7
	10	0 ± 0.0[d]			
	20	0 ± 0.0[d]			
Trial summary		Positive	Positive	Positive	Positive
Positive control[c]		566 ± 5.8	639 ± 3.7	583 ± 10.5	626 ± 6.1
TA98	0.000	23 ± 4.4	12 ± 1.2	13 ± 1.9	23 ± 1.8
	0.033		132 ± 42.8	20 ± 0.9	26 ± 1.2
	0.10		377 ± 30.5	19 ± 0.3	34 ± 1.7
	0.33	992 ± 161.0	642 ± 31.3	29 ± 0.7	38 ± 3.3
	1.0	1,540 ± 32.1	1,329 ± 101.3	52 ± 8.2	85 ± 11.5
	3.3	1,125 ± 174.3	1,086 ± 40.6	70 ± 6.6	238 ± 21.1
	10	370 ± 28.9			
	20	274 ± 22.4			
Trial summary		Positive	Positive	Positive	Positive
Positive control		106 ± 6.0	118 ± 19.4	259 ± 16.0	186 ± 9.0

[a] Study was performed at BioReliance Corporation (Rockville, MD). The detailed protocol is presented by Zeiger *et al.* (1992). 0 μg/plate was the solvent control (dimethylformamide).
[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] Slight toxicity

TABLE E7
Mutagenicity of 9-Nitroanthracene in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9 Trial 1	-S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	164 ± 4.2	177 ± 9.1	151 ± 9.8	185 ± 13.6
	100	235 ± 9.8	222 ± 11.8	325 ± 26.8	334 ± 6.4
	333	258 ± 9.9	229 ± 8.1	264 ± 11.1	352 ± 17.5
	1,000	254 ± 36.3[d]	259 ± 29.6[d]	373 ± 37.0[d]	398 ± 15.2[d]
	3,333	262 ± 41.1[e]	251 ± 11.1[e]	642 ± 36.0[e]	492 ± 56.6[e]
	6,667	283 ± 20.2[e]	297 ± 17.2[e]	565 ± 32.7[e]	736 ± 25.9[e]
Trial summary		Weakly positive	Weakly positive	Positive	Positive
Positive control[c]		669 ± 26.7	660 ± 25.6	714 ± 72.2	1,115 ± 77.7
TA98	0	14 ± 2.1	13 ± 1.5	14 ± 2.2	32 ± 3.4
	100	36 ± 4.1	54 ± 0.9	79 ± 13.7	128 ± 6.8
	333	40 ± 1.9	56 ± 6.2	116 ± 3.3	138 ± 9.3
	1,000	55 ± 3.2[d]	71 ± 6.2[d]	236 ± 22.0[d]	265 ± 35.2[d]
	3,333	86 ± 27.7[e]	82 ± 16.3[e]	432 ± 14.9[e]	478 ± 12.0[e]
	6,667	73 ± 7.0[e]	75 ± 2.7[e]	495 ± 26.8[e]	600 ± 41.7[e]
Trial summary		Positive	Positive	Positive	Positive
Positive control		115 ± 3.3	126 ± 4.1	478 ± 24.9	507 ± 29.0

[a] Study was performed at BioReliance Corporation (Rockville, MD). The detailed protocol is presented by Zeiger *et al.* (1992). 0 μg/plate was the solvent control (dimethylsulfoxide).
[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
[e] Background obscured by precipitate

TABLE E8
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with Anthraquinone by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]
Corn oil[d]	0	5	2.20 ± 0.51	
Anthraquinone	500	5	1.50 ± 0.32	0.8753
	1,000	5	1.30 ± 0.41	0.9361
	2,000	5	1.00 ± 0.32	0.9831
			P=0.982[e]	
Dimethylbenzanthracene[f]	12.5	5	8.20 ± 1.42	0.0000

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented in Shelby *et al.* (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control; dosed group values are significant at P≤0.008; positive control value is 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

TABLE E9
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Anthraquinone (99.9% Pure) in Feed for 14 Weeks[a]

Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male				
0	5	1.50 ± 0.32		3.5 ± 0.2
1,875	5	1.20 ± 0.25	0.7183	3.6 ± 0.5
3,750	5	1.00 ± 0.16	0.8415	3.9 ± 0.2
7,500	5	2.00 ± 0.45	0.1988	3.7 ± 0.2
15,000	5	2.00 ± 0.27	0.1988	4.4 ± 0.2
30,000	5	3.10 ± 0.37	0.0091	4.7 ± 0.5
		P<0.001[d]		
Female				
0	5	0.60 ± 0.29		2.3 ± 0.3
1,875	5	1.30 ± 0.20	0.0541	5.3 ± 0.4
3,750	5	1.70 ± 0.20	0.0109	4.6 ± 0.3
7,500	5	1.40 ± 0.24	0.0367	5.1 ± 0.6
15,000	5	1.60 ± 0.29	0.0165	4.7 ± 0.5
30,000	5	2.30 ± 0.30	0.0008	6.7 ± 0.2
		P=0.004		

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

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1 Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 14-Week Feed Study of Anthraquinone F-2
TABLE F2 Hematology Data for Mice in the 14-Week Feed Study of Anthraquinone F-9

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

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
Male						
Hematology						
n						
Day 4	9	9	10	10	10	9
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	40.2 ± 0.3	40.5 ± 0.4	41.1 ± 0.3	42.4 ± 0.5**	43.1 ± 0.3**	42.6 ± 0.8**
Day 22	46.1 ± 0.4	44.2 ± 0.4**	44.3 ± 0.5*	45.1 ± 0.4	45.0 ± 0.4	45.8 ± 0.2
Week 14	48.9 ± 0.6	44.5 ± 0.4**	45.6 ± 0.7**	44.9 ± 0.6**	44.8 ± 0.7**	46.1 ± 0.5
Hemoglobin (g/dL)						
Day 4	13.8 ± 0.1	13.8 ± 0.2	13.9 ± 0.2	14.5 ± 0.1**	14.6 ± 0.1**	14.5 ± 0.3**
Day 22	15.9 ± 0.1	15.3 ± 0.1**	15.3 ± 0.1*	15.5 ± 0.1	15.3 ± 0.1*	15.5 ± 0.1
Week 14	16.3 ± 0.1	14.9 ± 0.2**	15.1 ± 0.1**	14.7 ± 0.2**	14.7 ± 0.2**	14.9 ± 0.2**
Erythrocytes ($10^6/\mu L$)						
Day 4	6.39 ± 0.05	6.43 ± 0.06	6.56 ± 0.04*	6.74 ± 0.09**	6.94 ± 0.05**	6.85 ± 0.13**[b]
Day 22	7.55 ± 0.07	7.34 ± 0.07	7.25 ± 0.09*	7.34 ± 0.08	7.33 ± 0.08	7.34 ± 0.04
Week 14	9.13 ± 0.10	8.02 ± 0.09**	8.12 ± 0.11**	8.00 ± 0.09**	8.03 ± 0.15**	8.29 ± 0.10*
Reticulocytes ($10^6/\mu L$)						
Day 4	0.43 ± 0.05	0.43 ± 0.04	0.37 ± 0.04	0.35 ± 0.04	0.34 ± 0.03	0.36 ± 0.03
Day 22	0.22 ± 0.03	0.22 ± 0.02	0.21 ± 0.02	0.23 ± 0.02	0.25 ± 0.03	0.26 ± 0.02
Week 14	0.09 ± 0.01	0.15 ± 0.01**	0.15 ± 0.01**	0.15 ± 0.01**	0.15 ± 0.01**	0.17 ± 0.02**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 4	0.10 ± 0.02	0.13 ± 0.04	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.03	0.02 ± 0.01*
Day 22	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Week 14	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00	0.01 ± 0.01
Mean cell volume (fL)						
Day 4	62.9 ± 0.1	63.1 ± 0.3	62.8 ± 0.2	63.1 ± 0.3	62.4 ± 0.3	62.4 ± 0.2
Day 22	61.1 ± 0.2	60.2 ± 0.2	61.3 ± 0.2	61.4 ± 0.2	61.7 ± 0.2	62.4 ± 0.3**
Week 14	53.7 ± 0.3	55.7 ± 0.2**	56.2 ± 0.3**	56.3 ± 0.2**	56.0 ± 0.4**	55.8 ± 0.3**
Mean cell hemoglobin (pg)						
Day 4	21.6 ± 0.1	21.5 ± 0.1	21.2 ± 0.3	21.5 ± 0.2	21.0 ± 0.1*	21.1 ± 0.1*
Day 22	21.0 ± 0.1	20.9 ± 0.1	21.1 ± 0.1	21.1 ± 0.1	21.0 ± 0.1	21.1 ± 0.1
Week 14	17.9 ± 0.1	18.5 ± 0.1**	18.6 ± 0.1**	18.4 ± 0.1*	18.4 ± 0.2	18.0 ± 0.2
Mean cell hemoglobin concentration (g/dL)						
Day 4	34.2 ± 0.2	34.2 ± 0.2	33.8 ± 0.5	34.1 ± 0.3	33.9 ± 0.1	34.0 ± 0.2
Day 22	34.5 ± 0.1	34.7 ± 0.2	34.6 ± 0.1	34.4 ± 0.2	34.1 ± 0.1	33.8 ± 0.1**
Week 14	33.3 ± 0.2	33.4 ± 0.2	33.1 ± 0.2	32.8 ± 0.2	32.9 ± 0.2	32.3 ± 0.1**
Platelets ($10^3/\mu L$)						
Day 4	896.0 ± 58.5	846.4 ± 58.9	896.1 ± 23.1	971.2 ± 37.9	982.0 ± 53.4	817.6 ± 54.8
Day 22	805.5 ± 17.0	945.1 ± 9.3**	968.6 ± 11.3**	965.6 ± 13.9**	986.7 ± 13.5**	994.9 ± 19.4**
Week 14	668.3 ± 16.4	769.3 ± 21.4**	789.9 ± 9.4**	763.9 ± 15.1**	824.4 ± 20.0**	806.5 ± 24.5**
Leukocytes ($10^3/\mu L$)						
Day 4	9.18 ± 0.44	11.30 ± 0.75	9.68 ± 0.54	10.00 ± 0.71	10.15 ± 0.66	9.44 ± 0.34
Day 22	11.43 ± 0.78	11.46 ± 0.49	11.12 ± 0.28	10.20 ± 0.68	10.23 ± 0.58	10.46 ± 0.45
Week 14	11.69 ± 0.51	12.92 ± 0.45	13.19 ± 0.76	14.00 ± 0.95	12.66 ± 0.84	12.99 ± 0.76
Segmented neutrophils ($10^3/\mu L$)						
Day 4	1.34 ± 0.09	1.17 ± 0.13	1.41 ± 0.19	1.17 ± 0.09	1.32 ± 0.18	1.16 ± 0.12
Day 22	1.19 ± 0.12	1.35 ± 0.12	1.16 ± 0.13	1.16 ± 0.11	1.19 ± 0.13	1.20 ± 0.09
Week 14	2.35 ± 0.23	1.95 ± 0.23	1.80 ± 0.21	2.54 ± 0.36	1.95 ± 0.18	2.59 ± 0.36

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

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
Male (continued)						
Hematology (continued)						
n						
Day 4	9	9	10	10	10	9
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Bands ($10^3/\mu L$)						
Day 4	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	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
Lymphocytes ($10^3/\mu L$)						
Day 4	7.59 ± 0.47	9.76 ± 0.70	7.82 ± 0.45	8.57 ± 0.65	8.50 ± 0.60	8.01 ± 0.35
Day 22	9.89 ± 0.72	9.85 ± 0.41	9.68 ± 0.26	8.79 ± 0.58	8.79 ± 0.50	9.03 ± 0.49
Week 14	9.06 ± 0.42	10.80 ± 0.29	11.19 ± 0.64	11.27 ± 0.70	10.53 ± 0.76	10.22 ± 0.64
Monocytes ($10^3/\mu L$)						
Day 4	0.22 ± 0.04	0.34 ± 0.05	0.43 ± 0.04*	0.23 ± 0.04	0.30 ± 0.07	0.28 ± 0.05
Day 22	0.30 ± 0.07	0.24 ± 0.06	0.27 ± 0.05	0.20 ± 0.04	0.23 ± 0.05	0.16 ± 0.03
Week 14	0.17 ± 0.02	0.09 ± 0.03	0.07 ± 0.03	0.15 ± 0.04	0.10 ± 0.03	0.11 ± 0.05
Eosinophils ($10^3/\mu L$)						
Day 4	0.03 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.00 ± 0.00
Day 22	0.06 ± 0.03	0.02 ± 0.01	0.01 ± 0.01	0.04 ± 0.02	0.02 ± 0.02	0.07 ± 0.02
Week 14	0.11 ± 0.03	0.08 ± 0.03	0.13 ± 0.04	0.04 ± 0.03	0.08 ± 0.04	0.06 ± 0.03
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	16.8 ± 0.4	17.8 ± 0.5	17.2 ± 0.7	17.1 ± 0.4	17.9 ± 0.4	17.1 ± 0.4
Day 22	20.9 ± 0.4	22.3 ± 0.2**	22.7 ± 0.4**	23.1 ± 0.2**	22.1 ± 0.3**	23.0 ± 0.2**
Week 14	20.3 ± 0.4	21.1 ± 0.5	21.3 ± 0.3	22.1 ± 0.6**	22.3 ± 0.4**	22.4 ± 0.5**
Creatinine (mg/dL)						
Day 4	0.53 ± 0.02	0.60 ± 0.02**	0.59 ± 0.01**	0.61 ± 0.02**	0.67 ± 0.02**	0.64 ± 0.02**
Day 22	0.60 ± 0.00	0.71 ± 0.02**	0.70 ± 0.00**	0.69 ± 0.02**	0.72 ± 0.01**	0.71 ± 0.02**
Week 14	0.67 ± 0.02	0.71 ± 0.01*	0.73 ± 0.02**	0.73 ± 0.02**	0.76 ± 0.02**	0.74 ± 0.02**
Total protein (g/dL)						
Day 4	5.8 ± 0.1	5.7 ± 0.1	5.6 ± 0.1*	5.6 ± 0.1*	5.5 ± 0.1**	5.7 ± 0.1
Day 22	6.3 ± 0.1	6.8 ± 0.1**	6.9 ± 0.1**	7.2 ± 0.1**	7.2 ± 0.1**	7.3 ± 0.0**
Week 14	6.7 ± 0.1	7.2 ± 0.1**	7.3 ± 0.1**	7.4 ± 0.1**	7.5 ± 0.2**	7.8 ± 0.1**
Albumin (g/dL)						
Day 4	4.1 ± 0.0	4.1 ± 0.0	4.0 ± 0.0	4.0 ± 0.1*	4.0 ± 0.0*	4.1 ± 0.0
Day 22	4.5 ± 0.0	4.7 ± 0.0**	4.8 ± 0.0**	4.9 ± 0.1**	4.9 ± 0.1**	4.9 ± 0.0**
Week 14	4.7 ± 0.1	5.0 ± 0.1**	5.1 ± 0.1**	5.2 ± 0.1**	5.2 ± 0.1**	5.3 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 4	45 ± 2	51 ± 2	49 ± 2	53 ± 4*	60 ± 4**	64 ± 6**
Day 22	48 ± 3	40 ± 3	34 ± 1**	35 ± 1**	37 ± 2**	40 ± 2
Week 14	107 ± 9	63 ± 4**	62 ± 3**	91 ± 13	77 ± 9	118 ± 31

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

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Alkaline phosphatase (IU/L)						
Day 4	1,633 ± 33	1,649 ± 48	1,601 ± 37	1,686 ± 33	1,762 ± 28	1,634 ± 31
Day 22	1,207 ± 44	1,027 ± 23**	924 ± 11**	917 ± 15**	873 ± 17**	850 ± 16**
Week 14	624 ± 20	502 ± 16**	478 ± 14**	455 ± 19**	442 ± 21**	445 ± 17**
Creatine kinase (IU/L)						
Day 4	519 ± 70	598 ± 91	689 ± 175	629 ± 95	603 ± 94[c]	633 ± 95
Day 22	809 ± 217	410 ± 40	427 ± 64	394 ± 53	322 ± 24*	353 ± 36
Week 14	277 ± 29	318 ± 51	267 ± 77	299 ± 37	342 ± 48	305 ± 58
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 1	26 ± 2**	26 ± 1**	32 ± 4**	32 ± 2**	32 ± 3**
Day 22	21 ± 2	28 ± 1**	24 ± 1	25 ± 1	26 ± 2	26 ± 2
Week 14	43 ± 5	31 ± 3	29 ± 2	42 ± 6	36 ± 5	65 ± 21
Bile salts (μmol/L)						
Day 4	27.5 ± 3.2	42.5 ± 7.1*	35.1 ± 3.4	54.3 ± 5.4**	38.4 ± 5.3**	48.3 ± 5.5**
Day 22	24.6 ± 3.6	13.0 ± 2.2	14.7 ± 2.1	11.1 ± 1.6*	9.1 ± 1.4**	13.5 ± 1.5
Week 14	20.3 ± 0.8	13.5 ± 1.1**	10.7 ± 0.5**	13.5 ± 0.9**	11.6 ± 0.8**	14.7 ± 2.7**
Urinalysis						
n	10	10	10	10	10	10
Volume (mL/16 hr)						
Day 8	13.6 ± 0.5	12.1 ± 0.8	8.7 ± 0.8**	7.7 ± 0.9**	6.4 ± 1.0**	5.9 ± 0.9**
Day 26	16.1 ± 1.8	13.1 ± 2.0	13.8 ± 1.2	10.7 ± 1.5	16.1 ± 3.1	11.0 ± 0.9
Week 13	8.4 ± 1.1	9.0 ± 1.3	11.7 ± 2.0	10.7 ± 0.8	10.6 ± 0.8[c]	12.5 ± 1.3*
Specific gravity						
Day 8	1.008 ± 0.001	1.011 ± 0.001**	1.017 ± 0.002**	1.020 ± 0.002**	1.023 ± 0.003**	1.027 ± 0.004**
Day 26	1.014 ± 0.001	1.019 ± 0.002	1.017 ± 0.001	1.020 ± 0.001	1.019 ± 0.002	1.020 ± 0.002
Week 13	1.029 ± 0.002	1.030 ± 0.003	1.024 ± 0.002	1.027 ± 0.002	1.030 ± 0.001[c]	1.026 ± 0.002
Creatinine (mg/dL)						
Day 8	21.1 ± 3.1	22.0 ± 1.2	32.0 ± 4.1**	35.6 ± 3.6**	42.5 ± 5.7**	48.7 ± 8.5**
Day 26	33.2 ± 3.3	43.2 ± 5.4	35.5 ± 2.8	39.7 ± 2.7	34.8 ± 4.6	36.3 ± 3.1
Week 13	101.9 ± 8.6	93.0 ± 10.1	70.6 ± 7.5*	75.2 ± 5.5*	71.7 ± 7.1*	62.6 ± 4.8**
Glucose (μg/mg creatinine)						
Day 8	194 ± 8	207 ± 16	213 ± 10	188 ± 12	172 ± 16	199 ± 14
Day 26	203 ± 9	246 ± 8	278 ± 9**	260 ± 5**	250 ± 8*	224 ± 17
Week 13	140 ± 4	186 ± 5**	195 ± 12**	206 ± 8**	222 ± 10**	205 ± 9**
Protein (mg/mg creatinine)						
Day 8	2.83 ± 0.25	3.96 ± 0.28	4.01 ± 0.19*	4.39 ± 0.35**	3.89 ± 0.46	3.60 ± 0.41
Day 26	3.10 ± 0.07	6.00 ± 0.17**	6.78 ± 0.15**	6.99 ± 0.36**	5.81 ± 0.75**	7.82 ± 0.51**
Week 13	1.98 ± 0.06	3.62 ± 0.12**	3.98 ± 0.22**	4.28 ± 0.33**	3.40 ± 0.63**	4.74 ± 0.79**
Aspartate aminotransferase (mU/mg creatinine)						
Day 8	6 ± 1	10 ± 1	14 ± 3	9 ± 2	7 ± 2	8 ± 1
Day 26	10 ± 1	112 ± 23**	135 ± 15**	101 ± 6**	123 ± 10**	121 ± 6**
Week 13	12 ± 1	42 ± 4**	69 ± 8**	79 ± 19**	77 ± 6**	53 ± 7**
γ-Glutamyltransferase (IU/mg creatinine)						
Day 8	2.38 ± 0.15	2.06 ± 0.10	2.03 ± 0.11	1.95 ± 0.13*	1.65 ± 0.09**	1.55 ± 0.07**
Day 26	2.11 ± 0.12	2.40 ± 0.08	2.73 ± 0.10**	2.41 ± 0.10	2.38 ± 0.07	2.06 ± 0.06
Week 13	1.73 ± 0.07	1.57 ± 0.06	1.71 ± 0.06	1.49 ± 0.06*	1.37 ± 0.02**	1.38 ± 0.05**
N-acetyl-β-D-glucosaminidase (mU/mg creatinine)						
Day 8	16 ± 1	21 ± 1*	19 ± 1	18 ± 1	20 ± 1	19 ± 1
Day 26	13 ± 1	32 ± 3**	34 ± 3**	31 ± 2**	36 ± 3**	41 ± 2**
Week 13	9 ± 1	17 ± 1**	21 ± 1**	23 ± 4**	22 ± 1**	22 ± 1**

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

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
Female						
Hematology						
n						
Day 4	10	10	9	10	10	10
Day 22	10	10	10	10	9	9
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	42.3 ± 0.4	41.9 ± 0.5	43.1 ± 0.5	44.7 ± 0.6**	44.5 ± 0.7**	45.9 ± 0.5**
Day 22	47.2 ± 0.6	44.0 ± 0.3**	44.0 ± 0.3**	44.9 ± 0.3	45.1 ± 0.5	44.9 ± 0.5
Week 14	45.7 ± 0.7	42.6 ± 0.8**	44.0 ± 0.3	44.0 ± 0.5	44.2 ± 0.4	42.9 ± 0.6*
Hemoglobin (g/dL)						
Day 4	14.2 ± 0.1	14.1 ± 0.2	14.6 ± 0.1	15.1 ± 0.1**	15.0 ± 0.2**	15.3 ± 0.1**
Day 22	16.3 ± 0.2	14.8 ± 0.1**	14.9 ± 0.1**	15.0 ± 0.1**	15.2 ± 0.2	14.9 ± 0.2**
Week 14	15.4 ± 0.2	14.1 ± 0.2**	14.4 ± 0.1**	14.3 ± 0.2**	14.3 ± 0.1**	13.9 ± 0.2**
Erythrocytes ($10^6/\mu L$)						
Day 4	6.64 ± 0.08	6.64 ± 0.09	6.79 ± 0.09	7.09 ± 0.10**	7.07 ± 0.11**	7.26 ± 0.03**
Day 22	7.59 ± 0.10	6.83 ± 0.10**	6.92 ± 0.04**	7.05 ± 0.04**	7.07 ± 0.10*	7.07 ± 0.08*
Week 14	7.92 ± 0.10	7.06 ± 0.12**	7.41 ± 0.04	7.39 ± 0.07*	7.41 ± 0.07*	7.24 ± 0.11**
Reticulocytes ($10^6/\mu L$)						
Day 4	0.22 ± 0.01	0.28 ± 0.01	0.28 ± 0.02	0.27 ± 0.03	0.26 ± 0.02	0.25 ± 0.02
Day 22	0.13 ± 0.02	0.33 ± 0.05**	0.26 ± 0.02*	0.26 ± 0.02**	0.24 ± 0.02*	0.26 ± 0.02*
Week 14	0.10 ± 0.01	0.20 ± 0.02**	0.25 ± 0.02**	0.26 ± 0.02**	0.23 ± 0.01**	0.25 ± 0.02**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 4	0.08 ± 0.03	0.15 ± 0.06	0.12 ± 0.06	0.04 ± 0.02	0.01 ± 0.01	0.06 ± 0.04
Day 22	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.02	0.01 ± 0.01	0.00 ± 0.00
Week 14	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.01 ± 0.01
Mean cell volume (fL)						
Day 4	63.8 ± 0.2	63.2 ± 0.3	63.7 ± 0.3	63.1 ± 0.3	62.9 ± 0.2	63.3 ± 0.5
Day 22	62.2 ± 0.2	64.6 ± 1.1**	63.6 ± 0.3**	63.9 ± 0.1**	64.0 ± 0.3**	63.8 ± 0.3**
Week 14	57.7 ± 0.3	60.5 ± 0.2**	59.3 ± 0.2*	59.5 ± 0.2*	59.6 ± 0.3**	59.4 ± 0.2*
Mean cell hemoglobin (pg)						
Day 4	21.4 ± 0.1	21.3 ± 0.2	21.4 ± 0.1	21.3 ± 0.2	21.3 ± 0.2	21.1 ± 0.1
Day 22	21.5 ± 0.2	21.7 ± 0.3	21.6 ± 0.1	21.2 ± 0.1	21.5 ± 0.1	21.1 ± 0.2
Week 14	19.5 ± 0.1	19.9 ± 0.1	19.4 ± 0.1	19.3 ± 0.1	19.3 ± 0.1	19.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.6 ± 0.2	33.8 ± 0.2	33.7 ± 0.2	33.7 ± 0.3	33.8 ± 0.3	33.3 ± 0.2
Day 22	34.6 ± 0.2	33.7 ± 0.1**	34.0 ± 0.1**	33.3 ± 0.1**	33.7 ± 0.2**	33.3 ± 0.3**
Week 14	33.7 ± 0.3	33.0 ± 0.1	32.7 ± 0.1**	32.5 ± 0.1**	32.4 ± 0.2**	32.3 ± 0.2**
Platelets ($10^3/\mu L$)						
Day 4	958.0 ± 30.0	904.6 ± 23.6	915.7 ± 46.7	1,028.3 ± 23.5	996.6 ± 35.0	964.3 ± 32.9
Day 22	764.5 ± 29.7	830.6 ± 37.3	875.3 ± 15.3**	911.2 ± 16.0**	887.3 ± 38.9**	904.8 ± 19.6**
Week 14	740.6 ± 11.7	804.6 ± 12.0**	848.4 ± 14.6**	839.9 ± 10.7**	870.4 ± 11.2**	874.9 ± 18.8**
Leukocytes ($10^3/\mu L$)						
Day 4	9.22 ± 0.50	10.25 ± 0.60	9.52 ± 0.41	10.06 ± 0.63	10.22 ± 0.32	10.10 ± 0.44[c]
Day 22	9.11 ± 0.23	10.85 ± 0.81	10.37 ± 0.55	10.13 ± 0.77	10.10 ± 0.47	9.51 ± 0.65
Week 14	9.09 ± 0.41	10.05 ± 0.66	9.62 ± 0.32	8.57 ± 0.47	9.62 ± 0.72	10.08 ± 0.47
Segmented neutrophils ($10^3/\mu L$)						
Day 4	1.09 ± 0.11	1.41 ± 0.16	1.12 ± 0.20	1.05 ± 0.18	1.08 ± 0.12	1.31 ± 0.21[c]
Day 22	0.99 ± 0.09	1.53 ± 0.21	1.28 ± 0.27	1.00 ± 0.08	1.05 ± 0.16	0.84 ± 0.08
Week 14	1.87 ± 0.24	1.25 ± 0.19	1.28 ± 0.17	1.16 ± 0.19	1.25 ± 0.19	1.36 ± 0.20
Bands ($10^3/\mu L$)						
Day 4	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

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

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
Female (continued)						
Hematology (continued)						
n						
Day 4	10	10	9	10	10	10
Day 22	10	10	10	10	9	9
Week 14	10	10	10	10	10	10
Lymphocytes ($10^3/\mu L$)						
Day 4	7.90 ± 0.43	8.48 ± 0.49	8.23 ± 0.31	8.74 ± 0.56	8.90 ± 0.28	8.51 ± 0.43[c]
Day 22	7.89 ± 0.22	9.07 ± 0.68	8.88 ± 0.49	8.95 ± 0.77	8.90 ± 0.45	8.51 ± 0.61
Week 14	7.09 ± 0.26	8.70 ± 0.59*	8.22 ± 0.36	7.35 ± 0.34	8.23 ± 0.58	8.64 ± 0.32*
Monocytes ($10^3/\mu L$)						
Day 4	0.21 ± 0.05	0.25 ± 0.05	0.14 ± 0.04	0.24 ± 0.05	0.19 ± 0.05	0.22 ± 0.04
Day 22	0.20 ± 0.05	0.17 ± 0.06	0.18 ± 0.05	0.15 ± 0.05	0.12 ± 0.06	0.14 ± 0.04
Week 14	0.04 ± 0.02	0.06 ± 0.02	0.04 ± 0.02	0.02 ± 0.02	0.09 ± 0.02	0.04 ± 0.02
Eosinophils ($10^3/\mu L$)						
Day 4	0.02 ± 0.01	0.11 ± 0.04	0.03 ± 0.02	0.04 ± 0.01	0.06 ± 0.03	0.06 ± 0.03
Day 22	0.03 ± 0.02	0.08 ± 0.04	0.03 ± 0.02	0.04 ± 0.02	0.02 ± 0.02	0.02 ± 0.02
Week 14	0.09 ± 0.03	0.04 ± 0.02	0.08 ± 0.03	0.03 ± 0.02	0.06 ± 0.03	0.04 ± 0.02
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 22	10	10	10	10	9	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	19.9 ± 0.6	18.1 ± 0.8	17.0 ± 0.5**	18.3 ± 0.4	17.4 ± 0.5	18.3 ± 0.5
Day 22	22.2 ± 0.6	20.2 ± 0.5	21.1 ± 0.4	20.3 ± 0.7	21.2 ± 0.5	22.9 ± 0.6
Week 14	18.6 ± 0.5	19.7 ± 0.6	20.6 ± 0.4*	18.5 ± 0.5	18.9 ± 0.4	19.5 ± 0.6
Creatinine (mg/dL)						
Day 4	0.58 ± 0.01	0.61 ± 0.01	0.61 ± 0.01	0.63 ± 0.02**	0.63 ± 0.02*	0.65 ± 0.02**
Day 22	0.63 ± 0.02	0.64 ± 0.02	0.68 ± 0.02	0.65 ± 0.02	0.66 ± 0.02	0.70 ± 0.02*
Week 14	0.66 ± 0.02	0.68 ± 0.01	0.70 ± 0.00*	0.72 ± 0.01**	0.70 ± 0.02*	0.71 ± 0.02*
Total protein (g/dL)						
Day 4	6.0 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.8 ± 0.0	5.9 ± 0.1	6.1 ± 0.1
Day 22	6.3 ± 0.0	6.7 ± 0.1**	7.0 ± 0.1**	7.5 ± 0.1**	7.6 ± 0.1**	7.9 ± 0.1**
Week 14	6.5 ± 0.1	7.2 ± 0.1**	7.5 ± 0.1**	7.9 ± 0.1**	7.9 ± 0.1**	8.1 ± 0.0**
Albumin (g/dL)						
Day 4	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.5 ± 0.1
Day 22	4.6 ± 0.0	4.9 ± 0.0**	5.0 ± 0.1**	5.2 ± 0.1**	5.3 ± 0.1**	5.5 ± 0.0**
Week 14	4.7 ± 0.1	5.1 ± 0.1**	5.4 ± 0.1**	5.5 ± 0.1**	5.6 ± 0.1**	5.7 ± 0.0**
Alanine aminotransferase (IU/L)						
Day 4	44 ± 1	46 ± 2	53 ± 3*	51 ± 2*	53 ± 3**	53 ± 2**
Day 22	41 ± 1	36 ± 1*	31 ± 1**	32 ± 1**	30 ± 1**	36 ± 1**
Week 14	51 ± 3	38 ± 1**	41 ± 1*	66 ± 9	47 ± 4	46 ± 3
Alkaline phosphatase (IU/L)						
Day 4	1,271 ± 21	1,172 ± 38	1,161 ± 30	1,057 ± 22**	1,106 ± 43*	1,179 ± 35
Day 22	845 ± 20	698 ± 22**	651 ± 11**	561 ± 15**	598 ± 10**	625 ± 20**
Week 14	403 ± 20	330 ± 10**	321 ± 16**	293 ± 17**	282 ± 13**	274 ± 12**

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

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	10	10	10	10	10
Day 22	10	10	10	10	9	9
Week 14	10	10	10	10	10	10
Creatine kinase (IU/L)						
Day 4	669 ± 105	674 ± 99[c]	932 ± 183	855 ± 175	644 ± 74	661 ± 98
Day 22	353 ± 62	396 ± 36	317 ± 41	469 ± 86	227 ± 16	476 ± 145
Week 14	278 ± 25	248 ± 44	229 ± 27	327 ± 51	312 ± 45	230 ± 32
Sorbitol dehydrogenase (IU/L)						
Day 4	15 ± 1	27 ± 1**	32 ± 5**	31 ± 2**	30 ± 1**	30 ± 1**
Day 22	18 ± 1	22 ± 1**	22 ± 1*	21 ± 1	19 ± 1	20 ± 2
Week 14	23 ± 4	24 ± 2	26 ± 1	39 ± 4**	31 ± 1**	27 ± 2**
Bile salts (μmol/L)						
Day 4	20.0 ± 1.5	23.2 ± 1.5	34.3 ± 2.3**	32.7 ± 4.0**	31.5 ± 2.5*	30.4 ± 3.7**
Day 22	18.3 ± 2.0	18.7 ± 1.8	18.2 ± 2.2	17.7 ± 3.6	24.1 ± 4.0	23.2 ± 2.2
Week 14	26.4 ± 3.4	36.4 ± 6.6	35.8 ± 4.4	26.0 ± 2.5	20.0 ± 1.0	19.6 ± 2.1
Urinalysis						
n						
Day 8	10	10	10	10	10	10
Day 26	10	10	10	10	9	8
Week 13	10	10	10	10	10	10
Volume (mL/16 hr)						
Day 8	11 ± 1	11 ± 1	7 ± 1	8 ± 1	10 ± 1	11 ± 1[c]
Day 26	14 ± 2	12 ± 1	12 ± 1	16 ± 2	14 ± 1	8 ± 2[c]
Week 13	10 ± 1	10 ± 2	12 ± 2	17 ± 3	9 ± 2	9 ± 1
Specific gravity						
Day 8	1.012 ± 0.001	1.012 ± 0.002	1.016 ± 0.002	1.017 ± 0.003	1.015 ± 0.002	1.013 ± 0.001
Day 26	1.011 ± 0.001	1.011 ± 0.001	1.012 ± 0.001	1.011 ± 0.002	1.013 ± 0.002	1.020 ± 0.004
Week 13	1.016 ± 0.002	1.018 ± 0.003	1.018 ± 0.004	1.011 ± 0.002	1.022 ± 0.004	1.026 ± 0.004
Creatinine (mg/dL)						
Day 8	23.5 ± 3.1	20.8 ± 2.4	30.1 ± 4.6	27.8 ± 4.7	21.8 ± 3.4	19.9 ± 1.5
Day 26	26.0 ± 2.9	25.3 ± 2.2	24.9 ± 2.1	20.1 ± 3.1	20.8 ± 2.6	31.5 ± 6.6
Week 13	49.9 ± 5.6	52.4 ± 10.6	46.6 ± 9.3	25.7 ± 4.0*	53.3 ± 10.7	55.7 ± 9.8
Glucose (μg/mg creatinine)						
Day 8	209 ± 8	196 ± 15	185 ± 7	171 ± 19	165 ± 10*	178 ± 20
Day 26	183 ± 12	149 ± 10	161 ± 8	199 ± 15	183 ± 13	134 ± 8*
Week 13	127 ± 7	119 ± 5	111 ± 11	107 ± 9	143 ± 6	138 ± 10
Protein (mg/mg creatinine)						
Day 8	1.79 ± 0.09	1.69 ± 0.14	1.98 ± 0.17	2.04 ± 0.59	2.71 ± 0.90	2.69 ± 0.63
Day 26	1.13 ± 0.08	1.12 ± 0.07	1.47 ± 0.05	1.43 ± 0.18[c]	2.29 ± 0.67	2.15 ± 0.75
Week 13	0.93 ± 0.08	1.05 ± 0.06	0.89 ± 0.14	1.05 ± 0.10	1.61 ± 0.16**	2.47 ± 0.46**
Aspartate aminotransferase (mU/mg creatinine)						
Day 8	7 ± 1	16 ± 2**	22 ± 3**	23 ± 5**	39 ± 8**	25 ± 6**
Day 26	8 ± 7	4 ± 1	3 ± 1	5 ± 1	4 ± 2	1 ± 1
Week 13	3 ± 0	8 ± 0**	13 ± 1**	17 ± 1**	16 ± 1**	13 ± 1**

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

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
Female (continued)						
Urinalysis (continued)						
n						
Day 8	10	10	10	10	10	10
Day 26	10	10	10	10	9	8
Week 13	10	10	10	10	10	10
γ-Glutamyltransferase (IU/mg creatinine)						
Day 8	1.05 ± 0.05	1.65 ± 0.02**	0.75 ± 0.04*	0.98 ± 0.08	1.11 ± 0.03	1.03 ± 0.06
Day 26	0.84 ± 0.05	0.50 ± 0.03**	0.48 ± 0.01**	0.62 ± 0.03	0.64 ± 0.02	0.66 ± 0.03
Week 13	0.61 ± 0.05	0.40 ± 0.02**	0.44 ± 0.01*	0.46 ± 0.02	0.48 ± 0.04	0.59 ± 0.01
N-acetyl-β-D-glucosaminidase (mU/mg creatinine)						
Day 8	13 ± 1	19 ± 4	14 ± 1	18 ± 2	17 ± 2	18 ± 2
Day 26	15 ± 2	17 ± 2	16 ± 1	21 ± 3	22 ± 2*	17 ± 2
Week 13	15 ± 2	21 ± 2**	31 ± 2**	41 ± 4**	35 ± 4**	32 ± 1**

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

TABLE F2
Hematology Data for Mice in the 14-Week Feed Study of Anthraquinone[a]

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
Male						
n	10	10	10	9	10	10
Hematocrit (%)	55.6 ± 1.2	55.2 ± 1.1	53.3 ± 0.7	52.4 ± 0.9	52.5 ± 1.3	49.4 ± 1.2**
Hemoglobin (g/dL)	17.8 ± 0.2	17.9 ± 0.3	17.3 ± 0.2	17.1 ± 0.2	17.4 ± 0.3	16.7 ± 0.3*
Erythrocytes ($10^6/\mu L$)	11.45 ± 0.28	11.23 ± 0.25	10.79 ± 0.16	10.63 ± 0.17*	10.70 ± 0.29*	9.98 ± 0.28**
Reticulocytes ($10^6/\mu L$)	0.12 ± 0.02	0.17 ± 0.02	0.17 ± 0.01*	0.16 ± 0.01	0.20 ± 0.02**	0.20 ± 0.03**
Nucleated erythrocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	48.6 ± 0.2	49.3 ± 0.2	49.5 ± 0.2	49.2 ± 0.2	49.0 ± 0.2	49.6 ± 0.2*
Mean cell hemoglobin (pg)	15.6 ± 0.2	16.0 ± 0.2	16.1 ± 0.2	16.1 ± 0.1	16.3 ± 0.2*	16.8 ± 0.2*
Mean cell hemoglobin concentration (g/dL)	32.0 ± 0.3	32.5 ± 0.4	32.5 ± 0.2	32.7 ± 0.3	33.3 ± 0.2**	33.8 ± 0.3**
Platelets ($10^3/\mu L$)	844.1 ± 34.4	888.8 ± 35.6	951.5 ± 53.6	896.1 ± 34.0	1,000.9 ± 53.8*	1,005.5 ± 26.5**
Leukocytes ($10^3/\mu L$)	5.84 ± 0.36	4.64 ± 0.36	4.99 ± 0.58	5.42 ± 0.43	7.06 ± 0.71	4.70 ± 0.33
Segmented neutrophils ($10^3/\mu L$)	0.77 ± 0.08	0.68 ± 0.06	0.60 ± 0.10	0.71 ± 0.14	0.78 ± 0.12	0.70 ± 0.09
Bands ($10^3/\mu L$)	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 ($10^3/\mu L$)	5.01 ± 0.32	3.94 ± 0.31	4.35 ± 0.52	4.69 ± 0.39	6.20 ± 0.60	3.96 ± 0.35
Monocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils ($10^3/\mu L$)	0.06 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	0.08 ± 0.02	0.03 ± 0.01
Female						
n	10	10	10	10	10	10
Hematocrit (%)	49.8 ± 0.3	48.3 ± 0.3**	46.7 ± 0.5**	47.8 ± 0.5**	46.6 ± 0.3**	45.6 ± 0.6**
Hemoglobin (g/dL)	16.6 ± 0.1	16.2 ± 0.1*	15.8 ± 0.1**	16.1 ± 0.1**	15.9 ± 0.2**	15.7 ± 0.1**
Erythrocytes ($10^6/\mu L$)	10.32 ± 0.05	9.77 ± 0.05**	9.46 ± 0.10**	9.64 ± 0.11**	9.44 ± 0.06**	9.09 ± 0.09**
Reticulocytes ($10^6/\mu L$)	0.10 ± 0.01	0.16 ± 0.02*	0.19 ± 0.02**	0.20 ± 0.02**	0.19 ± 0.02**	0.26 ± 0.02**
Nucleated erythrocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	48.2 ± 0.1	49.3 ± 0.2**	49.4 ± 0.2**	49.7 ± 0.2**	49.3 ± 0.2**	50.3 ± 0.3**
Mean cell hemoglobin (pg)	16.1 ± 0.1	16.6 ± 0.1**	16.7 ± 0.1**	16.7 ± 0.1**	16.9 ± 0.1**	17.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.2	33.7 ± 0.1	33.8 ± 0.2	33.7 ± 0.2	34.1 ± 0.2**	34.5 ± 0.2**
Platelets ($10^3/\mu L$)	889.2 ± 13.9	993.1 ± 16.5**	971.9 ± 14.2**	1,012.1 ± 31.9**	1,065.3 ± 18.5**	1,096.6 ± 18.3**
Leukocytes ($10^3/\mu L$)	3.32 ± 0.25	3.64 ± 0.26	3.76 ± 0.38	3.54 ± 0.19	3.51 ± 0.29	4.42 ± 0.29*
Segmented neutrophils ($10^3/\mu L$)	0.60 ± 0.09	0.57 ± 0.09	0.56 ± 0.06	0.56 ± 0.07	0.55 ± 0.08	0.75 ± 0.09
Bands ($10^3/\mu L$)	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 ($10^3/\mu L$)	2.68 ± 0.25	3.03 ± 0.20	3.16 ± 0.33	2.92 ± 0.18	2.88 ± 0.21	3.63 ± 0.25*
Monocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils ($10^3/\mu L$)	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.08 ± 0.03	0.04 ± 0.02

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

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

TABLE G1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Feed Study of Anthraquinone G-2
TABLE G2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 12-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone G-3
TABLE G3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Feed Study of Anthraquinone G-4

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Feed Study of Anthraquinone[a]

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	338 ± 5	349 ± 3	347 ± 5	331 ± 6	336 ± 6	322 ± 4*
Heart						
Absolute	1.102 ± 0.022	1.087 ± 0.021	1.070 ± 0.021	1.026 ± 0.023*	1.043 ± 0.017*	1.037 ± 0.019*
Relative	3.262 ± 0.048	3.118 ± 0.057	3.084 ± 0.048*	3.104 ± 0.030	3.104 ± 0.052	3.224 ± 0.049
R. Kidney						
Absolute	1.236 ± 0.027	1.382 ± 0.027**	1.381 ± 0.039**	1.388 ± 0.025**	1.454 ± 0.037**	1.460 ± 0.038**
Relative	3.660 ± 0.055	3.966 ± 0.068**	3.979 ± 0.087**	4.203 ± 0.057**	4.324 ± 0.092**	4.537 ± 0.088**
Liver						
Absolute	13.416 ± 0.378	16.820 ± 0.455**	18.567 ± 0.457**	18.845 ± 0.379**	20.936 ± 0.467**	22.316 ± 0.503**
Relative	39.712 ± 0.942	48.219 ± 1.008**	53.535 ± 1.136**	57.071 ± 0.982**	62.268 ± 1.053**	69.315 ± 0.755**
Lung						
Absolute	1.529 ± 0.052	1.734 ± 0.066*	1.636 ± 0.056	1.538 ± 0.026	1.592 ± 0.052	1.474 ± 0.048
Relative	4.518 ± 0.107	4.980 ± 0.193	4.714 ± 0.136	4.667 ± 0.123	4.733 ± 0.133	4.582 ± 0.138
R. Testis						
Absolute	1.464 ± 0.020	1.532 ± 0.026*	1.561 ± 0.019*	1.529 ± 0.020*	1.546 ± 0.016**	1.590 ± 0.023**
Relative	4.335 ± 0.039	4.396 ± 0.066	4.501 ± 0.039	4.636 ± 0.092**	4.605 ± 0.077**	4.948 ± 0.075**
Thymus						
Absolute	0.257 ± 0.009	0.249 ± 0.008	0.264 ± 0.012	0.244 ± 0.008	0.257 ± 0.007	0.259 ± 0.012
Relative	0.762 ± 0.025	0.715 ± 0.021	0.760 ± 0.029	0.737 ± 0.018	0.762 ± 0.012	0.806 ± 0.036
Female						
Necropsy body wt	204 ± 3	198 ± 4	186 ± 3**	182 ± 2**	183 ± 3**	174 ± 1**
Heart						
Absolute	0.710 ± 0.018	0.729 ± 0.018	0.728 ± 0.030	0.693 ± 0.010	0.712 ± 0.010	0.691 ± 0.018
Relative	3.484 ± 0.085	3.674 ± 0.061	3.912 ± 0.144**	3.812 ± 0.057**	3.885 ± 0.067**	3.974 ± 0.105**
R. Kidney						
Absolute	0.708 ± 0.008	0.807 ± 0.012**	0.814 ± 0.011**	0.792 ± 0.019**	0.830 ± 0.017**	0.850 ± 0.015**
Relative	3.476 ± 0.040	4.074 ± 0.048**	4.378 ± 0.032**	4.347 ± 0.068**	4.526 ± 0.071**	4.891 ± 0.076**
Liver						
Absolute	6.431 ± 0.142	8.968 ± 0.199**	10.068 ± 0.217**	10.949 ± 0.195**	11.392 ± 0.296**	13.015 ± 0.207**
Relative	31.569 ± 0.612	45.272 ± 0.855**	54.202 ± 1.282**	60.189 ± 0.842**	62.101 ± 1.221**	74.840 ± 1.011**
Lung						
Absolute	1.049 ± 0.028	1.160 ± 0.033*	1.055 ± 0.019	1.093 ± 0.027	1.045 ± 0.028	1.015 ± 0.033
Relative	5.150 ± 0.120	5.868 ± 0.202	5.680 ± 0.132**	6.005 ± 0.120**	5.694 ± 0.114**	5.835 ± 0.179**
Thymus						
Absolute	0.260 ± 0.009	0.257 ± 0.011	0.210 ± 0.010**	0.223 ± 0.008**	0.222 ± 0.006**	0.207 ± 0.008**
Relative	1.278 ± 0.050	1.293 ± 0.042	1.126 ± 0.043	1.226 ± 0.037	1.207 ± 0.019	1.189 ± 0.050

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

	0 ppm	3,750 ppm
n	5	5
Male		
Necropsy body wt	431 ± 15	441 ± 9
R. Kidney		
Absolute	1.639 ± 0.062	1.729 ± 0.034
Relative	3.817 ± 0.188	3.926 ± 0.092
L. Kidney		
Absolute	1.656 ± 0.064	1.717 ± 0.015
Relative	3.863 ± 0.227	3.900 ± 0.073
Liver		
Absolute	17.806 ± 0.900	22.626 ± 0.532**
Relative	41.307 ± 1.760	51.380 ± 1.428**
Female		
Necropsy body wt	269 ± 13	202 ± 2**
R. Kidney		
Absolute	0.886 ± 0.009	0.938 ± 0.016*
Relative	3.326 ± 0.161	4.651 ± 0.122**
L. Kidney		
Absolute	0.905 ± 0.016	0.939 ± 0.017
Relative	3.384 ± 0.113	4.659 ± 0.126**
Liver		
Absolute	8.867 ± 0.364	11.395 ± 0.538**
Relative	33.087 ± 1.243	56.571 ± 3.098**

* 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 G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Feed Study of Anthraquinone[a]

	0 ppm	1,875 ppm	3,750 ppm	7,500 ppm	15,000 ppm	30,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	38.7 ± 0.9	39.8 ± 0.8	39.5 ± 1.0	39.6 ± 0.7	37.0 ± 0.6	37.8 ± 0.6
Heart						
Absolute	0.205 ± 0.010	0.199 ± 0.009	0.197 ± 0.010	0.239 ± 0.016	0.219 ± 0.011	0.221 ± 0.011
Relative	5.346 ± 0.336	5.015 ± 0.229	4.997 ± 0.234	6.026 ± 0.383	5.936 ± 0.309	5.837 ± 0.287
R. Kidney						
Absolute	0.293 ± 0.009	0.292 ± 0.005	0.311 ± 0.011	0.299 ± 0.007	0.293 ± 0.004	0.322 ± 0.005*
Relative	7.563 ± 0.122	7.355 ± 0.154	7.901 ± 0.336	7.548 ± 0.174	7.943 ± 0.174	8.518 ± 0.183**
Liver						
Absolute	1.723 ± 0.044	1.977 ± 0.049**	2.099 ± 0.060**	2.355 ± 0.071**	2.563 ± 0.072**	3.032 ± 0.045**
Relative	44.558 ± 0.469	49.669 ± 0.456**	53.105 ± 0.546**	59.312 ± 0.965**	69.203 ± 0.947**	80.206 ± 0.862**
Lung						
Absolute	0.324 ± 0.023	0.353 ± 0.019	0.326 ± 0.021	0.308 ± 0.011	0.316 ± 0.016	0.319 ± 0.017
Relative	8.374 ± 0.562	8.879 ± 0.434	8.318 ± 0.606	7.788 ± 0.278	8.561 ± 0.435	8.438 ± 0.451
R. Testis						
Absolute	0.125 ± 0.002	0.125 ± 0.001	0.127 ± 0.003	0.129 ± 0.002	0.124 ± 0.003	0.135 ± 0.004*
Relative	3.247 ± 0.095	3.153 ± 0.077	3.229 ± 0.108	3.257 ± 0.071	3.359 ± 0.071	3.571 ± 0.129
Thymus						
Absolute	0.056 ± 0.004	0.059 ± 0.005	0.057 ± 0.004	0.059 ± 0.003	0.043 ± 0.002	0.054 ± 0.006
Relative	1.459 ± 0.114	1.475 ± 0.105	1.437 ± 0.100	1.491 ± 0.078	1.174 ± 0.052	1.407 ± 0.127
Female						
Necropsy body wt	29.8 ± 0.6	32.3 ± 0.8	31.3 ±1.0	31.6 ± 0.8	31.2 ± 0.5	30.3 ± 0.8
Heart						
Absolute	0.131 ± 0.003	0.148 ± 0.004*	0.139 ± 0.004	0.141 ± 0.004	0.144 ± 0.004	0.145 ± 0.004*
Relative	4.399 ± 0.090	4.597 ± 0.128	4.450 ± 0.114	4.475 ± 0.161	4.611 ± 0.144	4.807 ± 0.126
R. Kidney						
Absolute	0.193 ± 0.004	0.200 ± 0.005	0.201 ± 0.006	0.192 ± 0.004	0.204 ± 0.004	0.194 ± 0.005
Relative	6.497 ± 0.146	6.195 ± 0.142	6.435 ± 0.117	6.104 ± 0.151	6.565 ± 0.152	6.433 ± 0.188
Liver						
Absolute	1.196 ± 0.035	1.437 ± 0.040**	1.547 ± 0.055**	1.620 ± 0.032**	1.886 ± 0.053**	2.263 ± 0.068**
Relative	40.110 ± 1.029	44.497 ± 0.753*	49.332 ± 0.642**	51.365 ± 0.729**	60.412 ± 1.016**	74.799 ± 2.121**
Lung						
Absolute	0.197 ± 0.004	0.212 ± 0.007	0.206 ± 0.010	0.209 ± 0.011	0.211 ± 0.010	0.228 ± 0.009
Relative	6.618 ± 0.149	6.588 ± 0.202	6.604 ± 0.329	6.669 ± 0.415	6.807 ± 0.401	7.519 ± 0.218
Thymus						
Absolute	0.052 ± 0.003	0.062 ± 0.003	0.060 ± 0.004	0.055 ± 0.003	0.052 ± 0.003	0.053 ± 0.004
Relative	1.755 ± 0.104	1.905 ± 0.079	1.894 ± 0.109	1.742 ± 0.108	1.680 ± 0.096	1.746 ± 0.119

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

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1 Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Feed Study of Anthraquinone H-2
TABLE H2 Summary of Estrous Cycle Characterization for Female Rats in the 14-Week Feed Study of Anthraquinone H-2
TABLE H3 Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Feed Study of Anthraquinone H-3
TABLE H4 Summary of Estrous Cycle Characterization for Female Mice in the 14-Week Feed Study of Anthraquinone H-3

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

	0 ppm	7,500 ppm	15,000 ppm	30,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	338 ± 5	330 ± 6	336 ± 6	322 ± 4
L. cauda epididymis	0.1634 ± 0.0033	0.1640 ± 0.0082	0.1729 ± 0.0053	0.1616 ± 0.0081
L. epididymis	0.4550 ± 0.0063	0.4603 ± 0.0082	0.4582 ± 0.0066	0.4477 ± 0.0089
L. testis	1.5446 ± 0.0187	1.6046 ± 0.0224	1.6235 ± 0.0274*	1.6482 ± 0.0255**
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.31 ± 0.21	7.97 ± 0.22	8.26 ± 0.27	8.08 ± 0.19
Spermatid heads (10^7/testis)	12.85 ± 0.39	12.80 ± 0.41	13.37 ± 0.38	13.33 ± 0.41
Spermatid count (mean/10^{-4} mL suspension)	64.23 ± 1.95	64.00 ± 2.03	66.85 ± 1.89	66.63 ± 2.03
Epididymal spermatozoal measurements				
Motility (%)	66.96 ± 1.94	66.46 ± 1.10	63.32 ± 1.76	63.47 ± 1.54
Concentration (10^6/g cauda epididymal tissue)	363 ± 26	323 ± 28	398 ± 38	421 ± 48

* Significantly different (P≤0.05) from the control group by William's test
** P≤0.01
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (necropsy body, cauda epididymis, and epididymis 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 Anthraquinone[a]

	0 ppm	7,500 ppm	15,000 ppm	30,000 ppm
n	10	10	10	10
Necropsy body wt (g)	204 ± 3	182 ± 2**	183 ± 3**	174 ± 1**
Estrous cycle length (days)	4.55 ± 0.17	4.90 ± 0.15	5.40 ± 0.31*	6.15 ± 0.33**
Estrous stages (% of cycle)				
Diestrus	36.7	39.2	43.3	43.3
Proestrus	17.5	20.0	16.7	17.5
Estrus	23.3	22.5	21.7	20.8
Metestrus	22.5	18.3	18.3	18.3

* Significantly different (P≤0.05) from the control group by Shirley's test
** Significantly different (P≤0.01) from the control group by William's test (necropsy body weight) or Shirley's test (estrous cycle length)
[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 Anthraquinone[a]

	0 ppm	7,500 ppm	15,000 ppm	30,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	38.7 ± 0.9	39.6 ± 0.7	37.0 ± 0.6	37.8 ± 0.6
L. cauda epididymis	0.0155 ± 0.0009	0.0154 ± 0.0009	0.0156 ± 0.0007	0.0164 ± 0.0005
L. epididymis	0.0424 ± 0.0013	0.0457 ± 0.0020	0.0430 ± 0.0019	0.0442 ± 0.0010
L. testis	0.1187 ± 0.0019	0.1202 ± 0.0009	0.1182 ± 0.0024	0.1236 ± 0.0021
Spermatid measurements				
Spermatid heads (10^7/g testis)	14.55 ± 0.49	14.08 ± 0.37	14.90 ± 0.63	15.37 ± 0.51
Spermatid heads (10^7/testis)	1.72 ± 0.06	1.69 ± 0.05	1.75 ± 0.06	1.90 ± 0.07
Spermatid count (mean/10^{-4} mL suspension)	53.90 ± 1.92	52.90 ± 1.48	54.73 ± 1.85	59.33 ± 2.09
Epididymal spermatozoal measurements				
Motility (%)	63.60 ± 1.11	64.03 ± 1.54	64.39 ± 0.68	62.89 ± 0.87
Concentration (10^6/g cauda epididymal tissue)	694 ± 185	647 ± 73	552 ± 62	651 ± 113

[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (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 Anthraquinone[a]

	0 ppm	7,500 ppm	15,000 ppm	30,000 ppm
n	10	10	10	10
Necropsy body wt (g)	29.8 ± 0.6	31.6 ± 0.8	31.2 ± 0.5	30.3 ± 0.8
Estrous cycle length (days)	4.28 ± 0.22[b]	4.75 ± 0.52	4.17 ± 0.17[b]	4.00 ± 0.00
Estrous stages (% of cycle)				
Diestrus	35.8	30.8	39.2	31.7
Proestrus	20.8	23.3	16.7	25.0
Estrus	22.5	24.2	20.0	21.7
Metestrus	20.8	21.7	24.2	21.7

[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 (necropsy 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.
[b] Estrous cycle was longer than 12 days or was unclear in 1 of 10 animals.

APPENDIX I
PHARMACOKINETIC MODEL
AND TOXICOKINETIC RESULTS

PHARMACOKINETIC MASS BALANCE DIFFERENTIAL EQUATIONS I-2
TABLE I1 Plasma Concentrations of Anthraquinone in Male Rats Administered Anthraquinone in Feed for 8 Days I-4
TABLE I2 Toxicokinetic Parameters in Male Rats Administered Anthraquinone in Feed for 8 Days I-4
FIGURE I1 Plasma Concentrations of Anthraquinone in Male Rats Administered Anthraquinone in Feed for 8 Days I-5
TABLE I3 Plasma Concentrations of Anthraquinone in Rats at the 3-, 6-, 12-, and 18-Month Interim Evaluations in the 2-Year Feed Study of Anthraquinone I-6
TABLE I4 Toxicokinetic Parameters in Rats at the 3-, 6-, 12-, and 18-Month Interim Evaluations in the 2-Year Feed Study of Anthraquinone I-8
FIGURE I2 Plasma Concentrations of Anthraquinone in Rats at the 3-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone I-9
FIGURE I3 Plasma Concentrations of Anthraquinone in Rats at the 6-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone I-10
FIGURE I4 Plasma Concentrations of Anthraquinone in Rats at the 12-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone I-11
FIGURE I5 Plasma Concentrations of Anthraquinone in Rats at the 18-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone I-12
TABLE I5 Plasma Concentrations of Anthraquinone in Aged Rats after a Single Gavage Dose of 100 mg/kg Anthraquinone I-13
TABLE I6 Toxicokinetic Parameters in Aged Rats after a Single Gavage Dose of 100 mg/kg Anthraquinone I-13
FIGURE I6 Plasma Concentrations of Anthraquinone in Aged Rats after a Single Gavage Dose of 100 mg/kg Anthraquinone I-14
TABLE I7 Plasma Concentrations of Anthraquinone in Male Mice Administered Anthraquinone in Feed for 8 Days I-15
TABLE I8 Toxicokinetic Parameters in Male Mice Administered Anthraquinone in Feed for 8 Days I-15
FIGURE I7 Plasma Concentrations of Anthraquinone in Male Mice Administered Anthraquinone in Feed for 8 Days I-16
TABLE I9 Plasma Concentrations of Anthraquinone in Mice at the 12-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone I-17
TABLE I10 Toxicokinetic Parameters in Mice at the 12-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone I-17
FIGURE I8 Plasma Concentrations of Anthraquinone in Mice at the 12-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone I-18
TABLE I11 Plasma Concentrations of Anthraquinone in Aged Mice after a Single Gavage Dose of 200 mg/kg Anthraquinone I-19
FIGURE I9 Plasma Concentrations of Anthraquinone in Aged Mice after a Single Gavage Dose of 200 mg/kg Anthraquinone I-20
TABLE I12 Cardiac Output, Organ Volumes, and Organ Blood Perfusion Rates of Rats for the Physiologically Based Pharmacokinetic Model of Anthraquinone I-21

PHYSIOLOGICALLY BASED PHARMACOKINETIC MASS BALANCE MODEL DIFFERENTIAL EQUATIONS

Below are the mass balance differential equations that represent, in quantitative terms, the physiological and biochemical processes that affect the behavior of anthraquinone in exposed rats. Definition of each abbreviation used in the equations is also listed below.

Non-metabolizing tissue concentration:

$$\frac{dC_{tissue}}{dt} = \frac{Q_{tissue}\,Perm(C_{cap} - \frac{C_{tissue}}{PC_{tissue}})}{V_{tissue}}$$

$$\frac{dC_{cap}}{dt} = \frac{Q_{tissue}\,Perm(\frac{C_{tissue}}{PC_{tissue}} - C_{cap})}{V_{tissue}} + \frac{Q_{tissue}(C_{art} - C_{cap})}{V_{cap}}$$

Metabolism:

$$Saturable = \frac{V_{liver} \bullet V_{max} \bullet C_{liver}}{k_m + C_{liver}}$$

Liver concentration:

$$\frac{dC_{liver}}{dt} = \frac{(Q_{liver} + Q_{GItract})Perm(C_{livercap} - \frac{C_{liver}}{PC_{liver}})}{V_{liver}} - Saturable - k_{bile} \bullet V_{liver} \bullet C_{liver}$$

$$\frac{dC_{livercap}}{dt} = \frac{Q_{liver}\,Perm(\frac{C_{liver}}{PC_{liver}} - C_{livercap})}{V_{liver}} + \frac{Q_{stomach}C_{stomachcap}}{V_{stomachcap}} +$$

$$\frac{Q_{intestine}C_{intestinecap}}{V_{intestinecap}} + \frac{Q_{colon}C_{coloncap}}{V_{coloncap}} - \frac{(Q_{liver} + Q_{stomach} + Q_{intestine} + Q_{colon})C_{livercap}}{V_{livercap}}$$

Plasma concentration:

$$\frac{dC_{plasma}}{dt} = \frac{\left(\sum_{tissues}(Q_{tissue}C_{cap}) - C_{plasma}\sum_{tissues}Q_{tissue}\right)}{V_{plasma}} + \frac{V_{abs} \bullet A_{jejunum}}{k_{abs} + A_{jejunum}}$$

Oral administration:

$$\frac{dC_{stomach\ lumen}}{dt} = -\frac{-Q_{stomach\ lumen}C_{stomach\ lumen}}{V_{stomach\ lumen}}$$

$$\frac{dC_{duodenum\ lumen}}{dt} = \frac{Q_{stomach\ lumen}C_{stomach\ lumen}}{V_{stomach\ lumen}} - \frac{Q_{duodenum\ lumen}C_{duodenum\ lumen}}{V_{duodenum\ lumen}} + k_{bile} \bullet C_{liver} \bullet V_{liver}$$

$$\frac{dC_{jejunum\ lumen}}{dt} = \frac{Q_{duodenum\ lumen}C_{duodenum\ lumen}}{V_{duodenum\ lumen}} - \frac{Q_{jejunum\ lumen}C_{jejunum\ lumen}}{V_{jejunum\ lumen}} + \frac{V_{abs}C_{jejunum\ lumen}}{k_{abs} + C_{jejunum\ lumen}}$$

$$\frac{dC_{jejunum\ lumen}}{dt} = \frac{Q_{jejunum\ lumen}C_{jejunum\ lumen}}{V_{jejunum\ lumen}} - \frac{Q_{colon\ lumen}C_{colon\ lumen}}{V_{colon\ lumen}}$$

Definition of Abreviations

Concentration:
C_{tissue} concentration in tissue space (mg/kg)
C_{plasma} concentration in plasma (mg/L)

Flow:
Q_{tissue} blood flow to tissue
Q_{lumen} chyme flow

Partition coefficient and permeability constant:
PC_{tissue} tissue/plasma partition coefficient
Perm capillary permeability constant

Volume:
V_{tissue} volume of tissue

Absorption, metabolism, and elimination rates:
V_{max} maximum velocity of saturable metabolism (mM/hr)
K_m Michaelis-Menten constant for metabolism (mM)
V_{abs} maximum velocity of saturable absorption (mM/hr)
K_{abs} Michaelis-Menten constant for metabolism (mM)
K_{bile} linear bile transfer constant (hr^{-1})

TABLE I1
Plasma Concentrations of Anthraquinone in Male Rats Administered Anthraquinone in Feed for 8 Days[a]

	469 ppm	938 ppm	1,875 ppm	3,750 ppm
n	3	3	3	3
Time of collection				
0800	0.357 ± 0.045	0.666 ± 0.041	0.796 ± 0.077	1.585 ± 0.293
1000	0.213 ± 0.017	0.680 ± 0.066	0.720 ± 0.001	1.067 ± 0.164
1200	0.236 ± 0.033	0.526 ± 0.043	0.553 ± 0.108	1.489 ± 0.149
1400	0.235 ± 0.017	0.538 ± 0.045	0.574 ± 0.019	0.990 ± 0.036
1600	0.262 ± 0.029	0.497 ± 0.039	0.558 ± 0.022	0.688 ± 0.051
1800	0.215 ± 0.028	0.578 ± 0.075	0.600 ± 0.052	1.246 ± 0.296
2000	0.215 ± 0.015	0.574 ± 0.052	0.708 ± 0.069	1.266 ± 0.153
2200	0.192 ± 0.011	0.719 ± 0.025	0.739 ± 0.058	1.247 ± 0.152
2400	0.195 ± 0.012	0.612 ± 0.055	0.752 ± 0.103	1.290 ± 0.140
0200	0.230 ± 0.013	0.558 ± 0.038	0.621 ± 0.054	1.229 ± 0.199
0400	0.274 ± 0.038	0.594 ± 0.065	0.651 ± 0.095	1.113 ± 0.059
0600	0.218 ± 0.034	0.499 ± 0.036	0.651 ± 0.037	1.086 ± 0.147

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

TABLE I2
Toxicokinetic Parameters in Male Rats Administered Anthraquinone in Feed for 8 Days[a]

Concentration (ppm)	C_{min} (μg/mL)	T_{min}	C_{max} (μg/mL)	T_{max}
469	0.192	2200	0.357	0800
938	0.497	1600	0.719	2000
1,875	0.553	1200	0.796	0800
3,750	0.688	1600	1.58	0800

[a] C_{min}=minimum mean concentration; T_{min}=time of minimum mean concentration; C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration

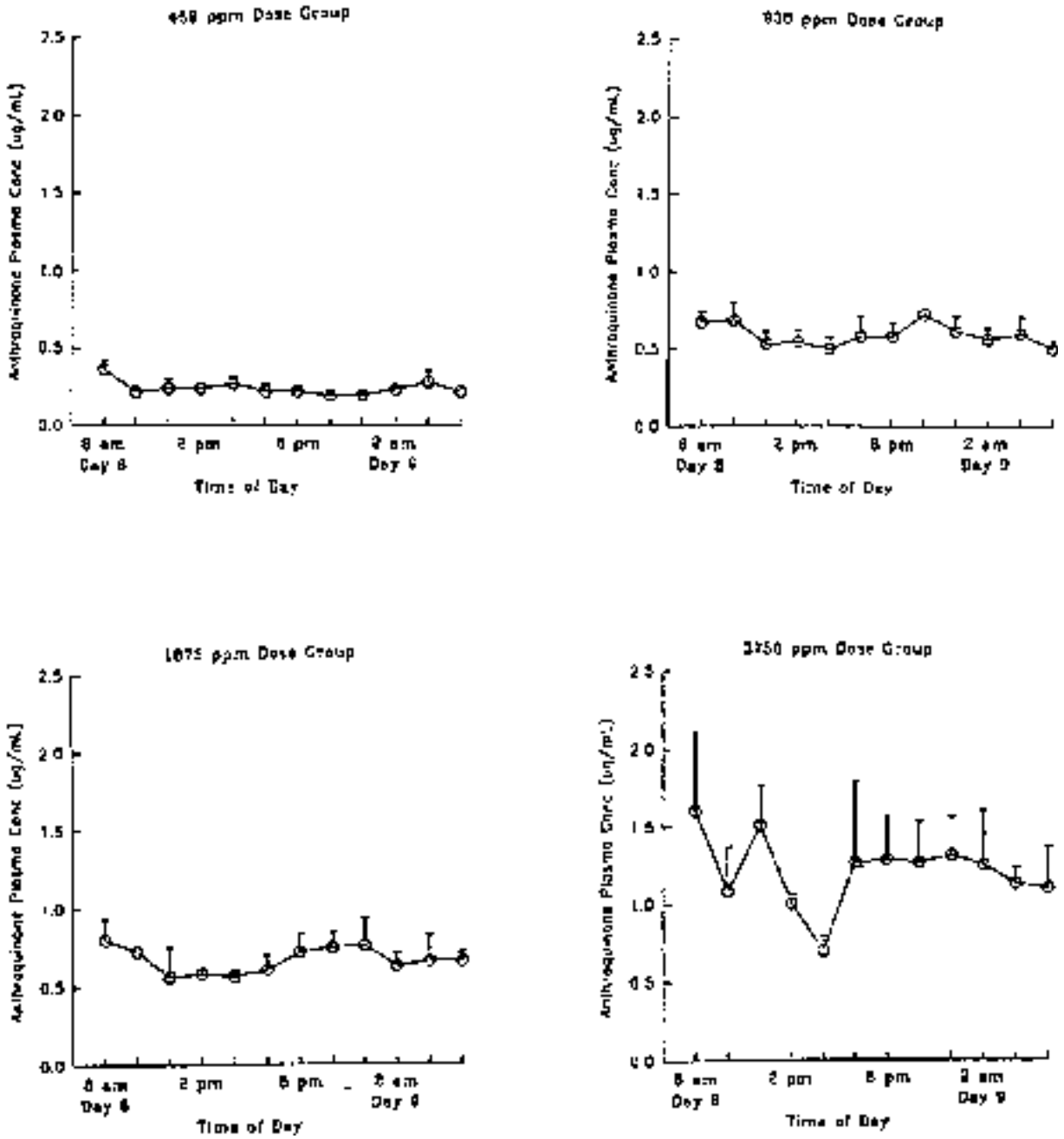

FIGURE I1
Plasma Concentrations of Anthraquinone in Male Rats Administered Anthraquinone in Feed for 8 Days

TABLE I3
Plasma Concentrations of Anthraquinone in Rats at the 3-, 6-, 12-, and 18-Month Interim Evaluations in the 2-Year Feed Study of Anthraquinone[a]

	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Male				
n	3	3	3	3
Month 3				
Time of collection				
0800	0.193 ± 0.090	0.701 ± 0.051	0.566 ± 0.037	0.836 ± 0.101
1130	0.255 ± 0.013	0.898 ± 0.138	0.587 ± 0.133	0.677 ± 0.074
1400	0.213 ± 0.012[b]	0.564 ± 0.036[b]	0.448 ± 0.103[b]	0.817 ± 0.235[b]
1730	0.192 ± 0.022	0.571 ± 0.051	0.554 ± 0.063	0.715 ± 0.146
2100	0.230 ± 0.019	0.621 ± 0.037	0.429 ± 0.104	0.826 ± 0.214
0030	0.234 ± 0.009	0.607 ± 0.080	0.515 ± 0.100	0.818 ± 0.045
0400	0.210 ± 0.024	0.564 ± 0.027[b]	0.527 ± 0.058	0.996 ± 0.072
n	2	2	2	2
Month 6				
Time of collection				
0600	0.232 ± 0.031	0.335 ± 0.051	0.638 ± 0.070	1.180 ± 0.025
1100	0.216 ± 0.018	0.279 ± 0.025	0.515 ± 0.021	1.103 ± 0.005
1600	0.240 ± 0.005	0.277 ± 0.036	0.655 ± 0.079	1.248 ± 0.239
2100	0.165 ± 0.011	0.344 ± 0.001	0.712 ± 0.163	0.850 ± 0.189
0200	0.252 ± 0.036	0.326 ± 0.027	0.637 ± 0.061	0.829 ± 0.044
Month 12				
Time of collection				
0600	0.326 ± 0.061	0.596 ± 0.043	0.538 ± 0.107	0.865 ± 0.090
1100	0.125 ± 0.058	0.669 ± 0.054	0.560 ± 0.013	0.840 ± 0.161
1600	0.357 ± 0.024	0.651 ± 0.008	0.546 ± 0.041	1.080 ± 0.091
2100	0.282 ± 0.005	0.476[c]	0.421 ± 0.014	1.023 ± 0.046
0200	0.284 ± 0.014	0.554[c]	0.495 ± 0.037	1.103 ± 0.041
Month 18				
Time of collection				
0600	0.425[c]	0.741 ± 0.101	0.900 ± 0.255	1.461 ± 0.216
1100	0.280 ± 0.024	0.701 ± 0.134	0.646 ± 0.084	1.384 ± 0.441
1600	0.355 ± 0.006	0.673 ± 0.083	0.627 ± 0.022	0.853 ± 0.068
2100	0.275 ± 0.001	0.692 ± 0.150	1.118 ± 0.571	1.043 ± 0.414
0200	0.391 ± 0.117	0.951 ± 0.062	1.061 ± 0.065	1.438 ± 0.158

TABLE I3
Plasma Concentrations of Anthraquinone in Rats at the 3-, 6-, 12-, and 18-Month Interim Evaluations in the 2-Year Feed Study of Anthraquinone

	469 ppm	938 ppm	1,875 ppm	3,750 ppm
Female				
n	3	3	3	3
Month 3				
Time of collection				
0800	0.654 ± 0.061	2.230 ± 0.209	2.340 ± 0.205	2.292 ± 0.438
1130	0.514 ± 0.013	2.180 ± 0.133	1.913 ± 0.287	2.703 ± 0.560
1400	0.595 ± 0.139[b]	1.935 ± 0.197[b]	1.516 ± 0.522[b]	2.051 ± 0.189[b]
1730	0.630 ± 0.045	2.029 ± 0.148	2.429 ± 0.449	2.383 ± 0.378
2100	0.783 ± 0.192	2.059 ± 0.220	1.988 ± 0.159	2.671 ± 0.291
0030	0.681 ± 0.053	2.727 ± 0.148	1.874 ± 0.187	3.524 ± 0.403
0400	0.933 ± 0.129	2.328 ± 0.271	2.066 ± 0.100	2.489 ± 0.453
n	2	2	2	2
Month 6				
Time of collection				
0600	0.393 ± 0.015	0.795 ± 0.137	1.523 ± 0.135	2.395 ± 0.606
1100	0.614 ± 0.152	1.333 ± 0.021	1.762 ± 0.336	3.797 ± 0.506
1600	0.451 ± 0.012	0.875 ± 0.046	1.914 ± 0.315	3.410 ± 0.783
2100	0.472 ± 0.001	0.997 ± 0.049	1.664 ± 0.385	2.521 ± 0.215
0200	0.466 ± 0.109	1.022 ± 0.209	1.953 ± 0.132	2.003 ± 0.258
Month 12				
Time of collection				
0600	0.666 ± 0.178	1.933 ± 0.230	1.697 ± 0.468	2.346 ± 0.094
1100	0.752 ± 0.030	2.743 ± 0.064	2.347 ± 0.165	2.955 ± 1.053
1600	0.809 ± 0.181	2.225 ± 0.206	2.653 ± 0.314	2.224 ± 0.438
2100	0.724 ± 0.085	2.294 ± 0.041	1.430 ± 0.069	1.192 ± 0.299
0200	0.829 ± 0.029	2.165 ± 0.325	1.752 ± 0.311	2.986 ± 0.250
Month 18				
Time of collection				
0600	0.689 ± 0.301	1.132 ± 0.172	1.896[c]	1.933 ± 0.068
1100	0.487 ± 0.057	1.539 ± 0.180	1.764 ± 0.123	2.856 ± 0.051
1600	0.928 ± 0.254	1.144 ± 0.069	2.415 ± 0.669	2.458 ± 0.371
2100	0.669 ± 0.018	1.527 ± 0.016	1.602 ± 0.345	1.213 ± 0.175
0200	0.454 ± 0.015	2.063 ± 0.284	1.882 ± 0.030	2.227 ± 0.203

[a] Data are given in μg/mL as the mean ± standard error.
[b] n=2
[c] n=1; no standard error calculated

TABLE I4
Toxicokinetic Parameters in Rats at the 3-, 6-, 12-, and 18-Month Interim Evaluations in the 2-Year Feed Study of Anthraquinone[a]

	Concentration (ppm)	C_{min} (μg/mL)	T_{min}	C_{max} (μg/mL)	T_{max}
Male					
Month 3	469	0.192	1730	0.255	1130
	938	0.564	1400	0.898	1130
	1,875	0.429	2100	0.588	1130
	3,750	0.677	1130	0.996	0400
Month 6	469	0.165	2100	0.252	0200
	938	0.277	1600	0.335	0600
	1,875	0.515	1100	0.712	2100
	3,750	0.829	0200	1.25	1600
Month 12	469	0.125	1100	0.357	1600
	938	0.476	2100	0.669	1100
	1,875	0.421	2100	0.560	1100
	3,750	0.840	1100	1.10	0200
Month 18	469	0.275	2100	0.425	0600
	938	0.673	1600	0.951	0200
	1,875	0.627	1600	1.12	2100
	3,750	0.853	1600	1.46	0600
Female					
Month 3	469	0.514	1130	0.933	0400
	938	1.94	1400	2.73	2430
	1,875	1.52	1400	2.43	1730
	3,750	2.05	1400	3.52	1230
Month 6	469	0.393	0600	0.614	1100
	938	0.795	0600	1.33	1100
	1,875	1.52	0600	1.95	0200
	3,750	2.00	0200	3.80	1100
Month 12	469	0.666	0600	0.829	0200
	938	1.93	0600	2.74	1100
	1,875	1.43	2100	2.65	1600
	3,750	1.19	2100	2.99	0200
Month 18	469	0.454	0200	0.928	1600
	938	1.13	0600	2.06	0200
	1,875	1.60	2100	2.42	1600
	3,750	1.21	2100	2.86	1100

[a] C_{min} = minimum mean concentration; T_{min} = time of minimum mean concentration; C_{max} = maximum mean concentration; T_{max} = time of maximum mean concentration

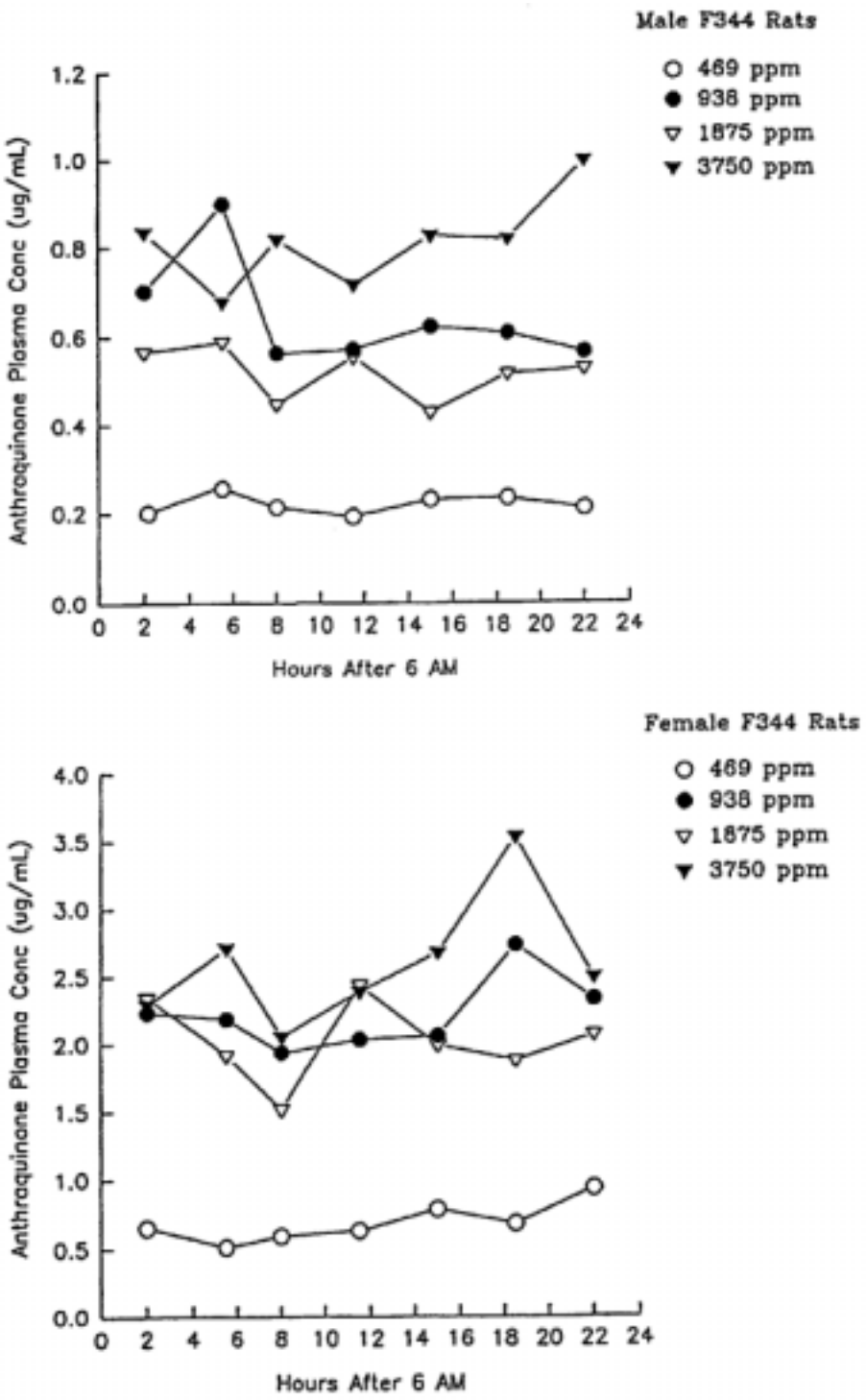

FIGURE I2
Plasma Concentrations of Anthraquinone in Rats at the 3-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone

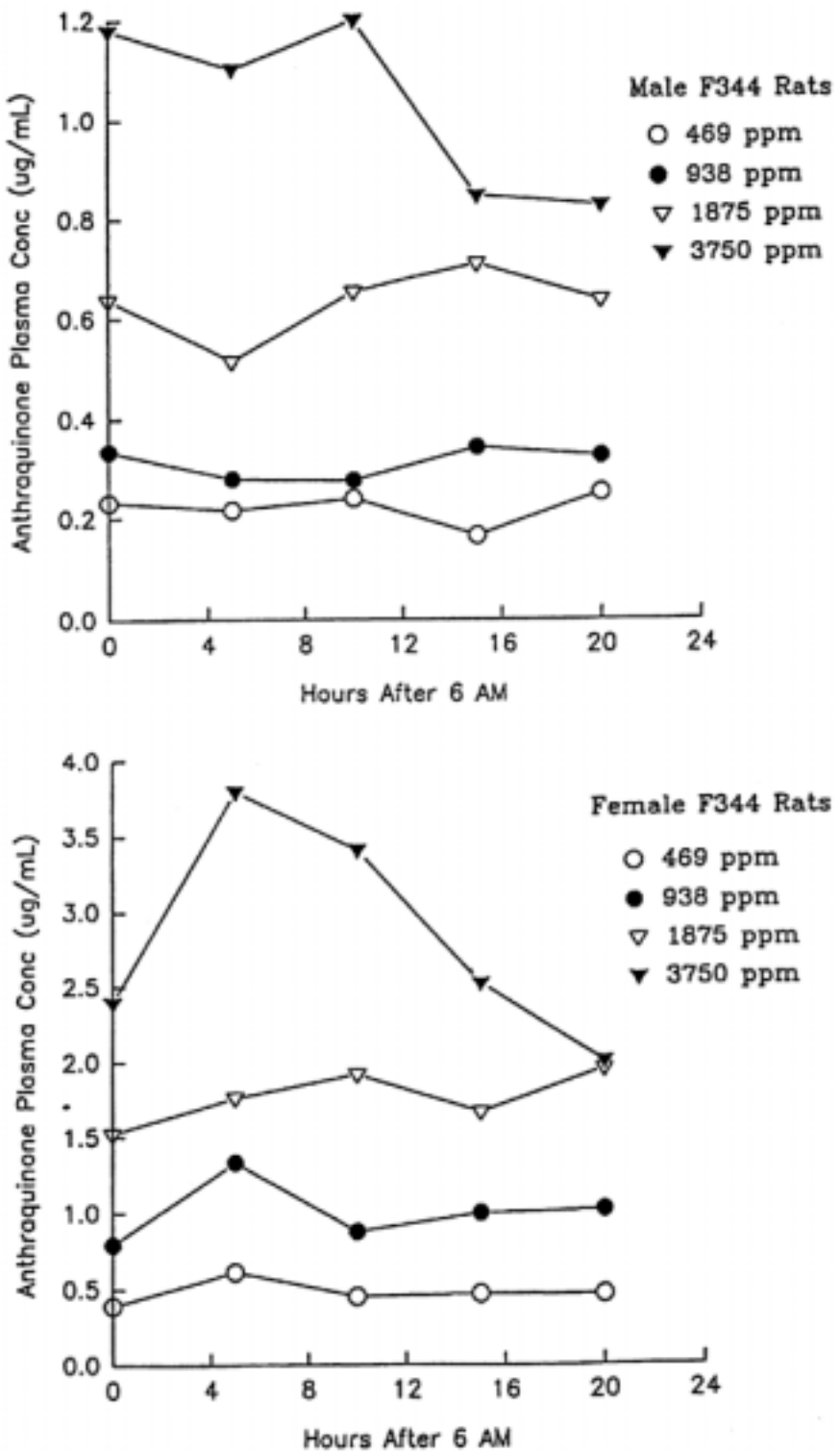

FIGURE I3
Plasma Concentrations of Anthraquinone in Rats at the 6-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone

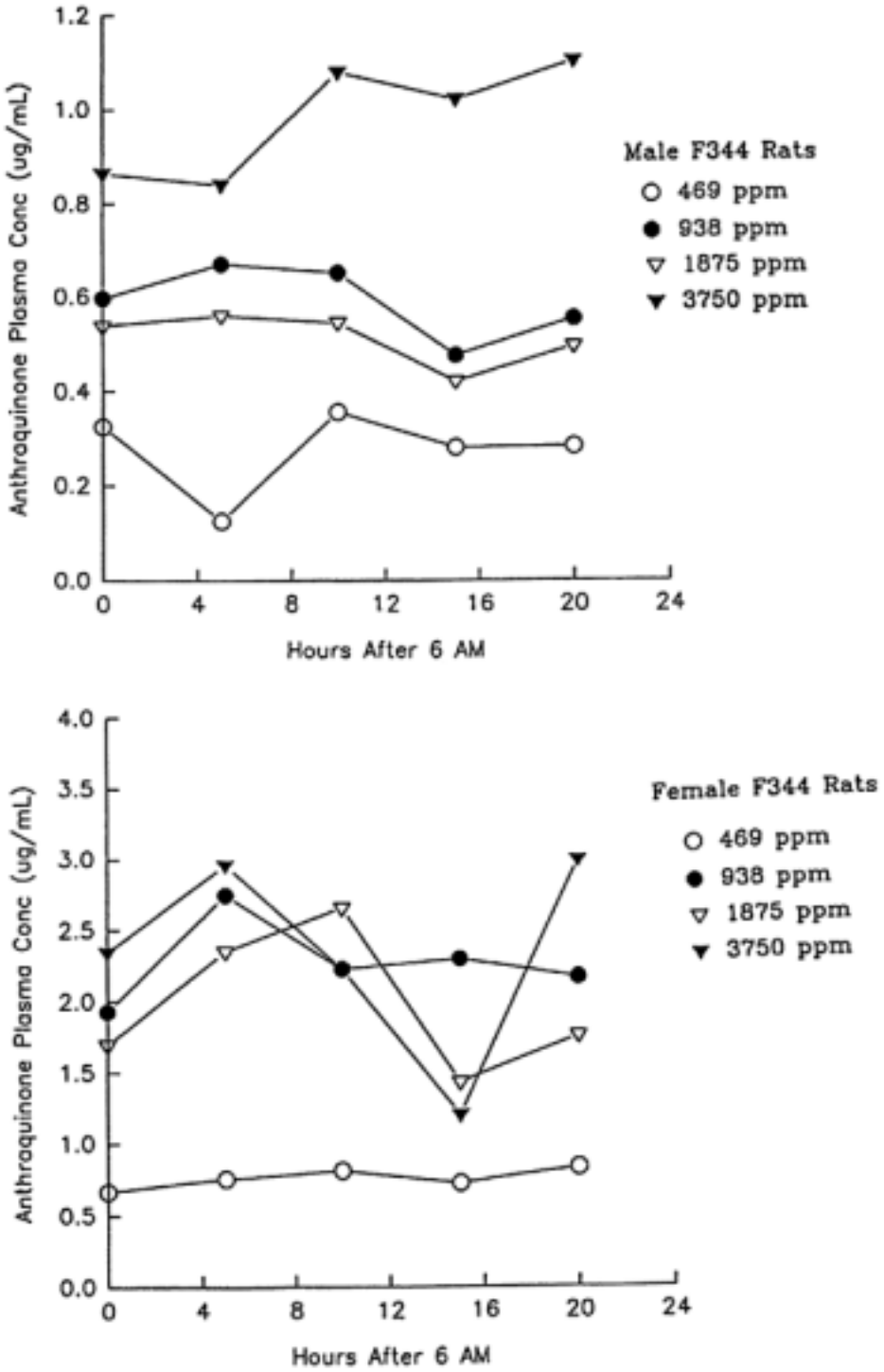

FIGURE I4
Plasma Concentrations of Anthraquinone in Rats at the 12-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone

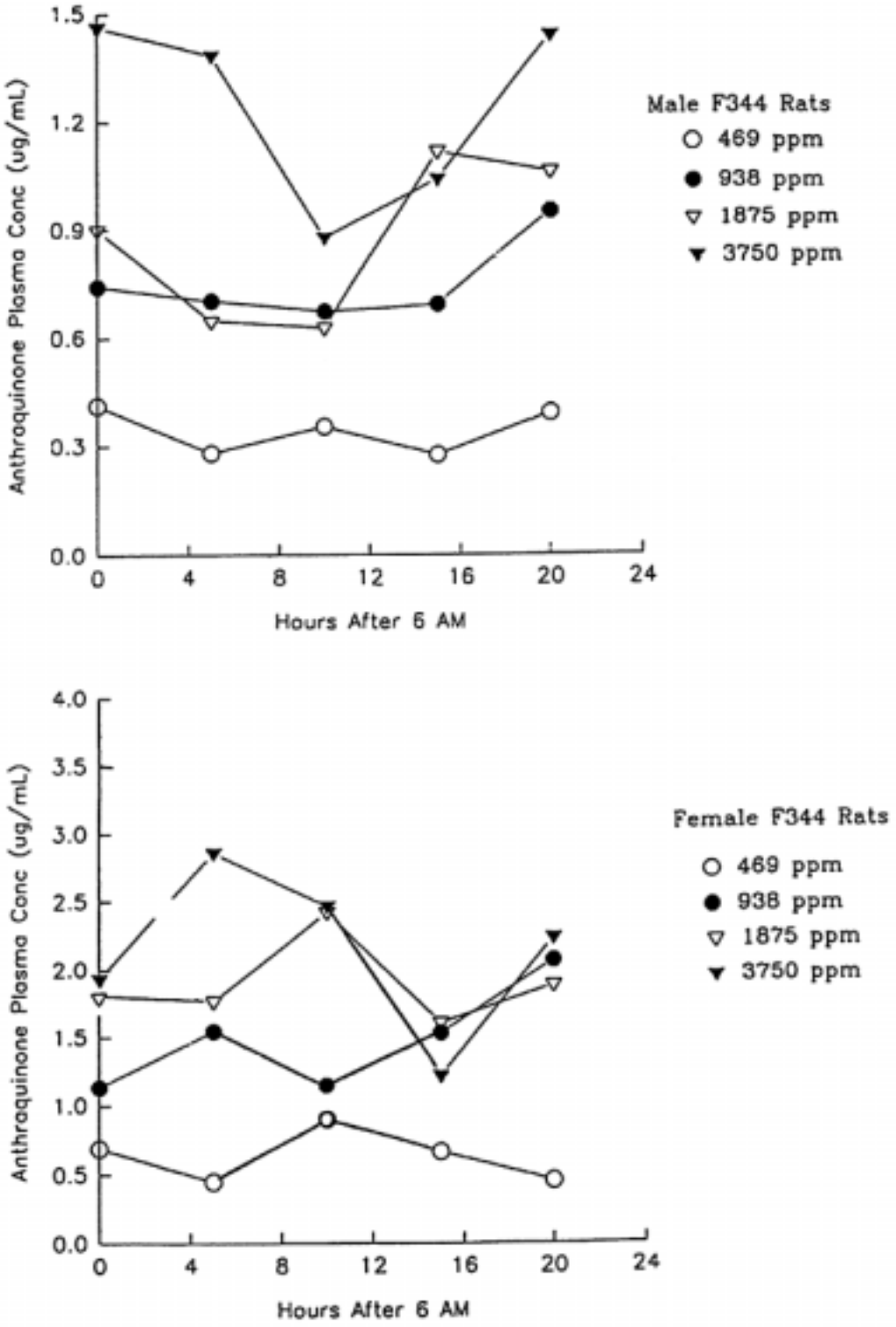

FIGURE I5
Plasma Concentrations of Anthraquinone in Rats at the 18-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone

TABLE I5
Plasma Concentrations of Anthraquinone in Aged Rats after a Single Gavage Dose of 100 mg/kg Anthraquinone[a]

Time after Dosing (hours)	Concentration[b] (μg/mL)
Male	
2	0.118 ± 0.007
6	0.373 ± 0.039
12	0.370 ± 0.096
24	0.486 ± 0.185
36	0.195 ± 0.064[c]
Female	
2	0.130 ± 0.008
6	0.339 ± 0.008
12	0.434 ± 0.014
24	0.283 ± 0.106
36	0.409 ± 0.163[c]

[a] Three animals were bled at each time point.
[b] Data are given in μg/mL as the mean ± standard error.
[c] Two animals bled

TABLE I6
Toxicokinetic Parameters in Aged Rats after a Single Gavage Dose of 100 mg/kg Anthraquinone[a]

	C_{max} (μg/mL)	T_{max} (hours)	$t_{½}$ (minutes)	AUC (μg/mL•minute)
Male	0.49	24	ND[b]	ND
Female	0.43	12	ND	ND

[a] C_{max} = maximum mean concentration; T_{max} = time of maximum mean concentration; $t_{½}$ = elimination half-life; AUC = area under the curve
[b] ND = Not determined due to insufficient data

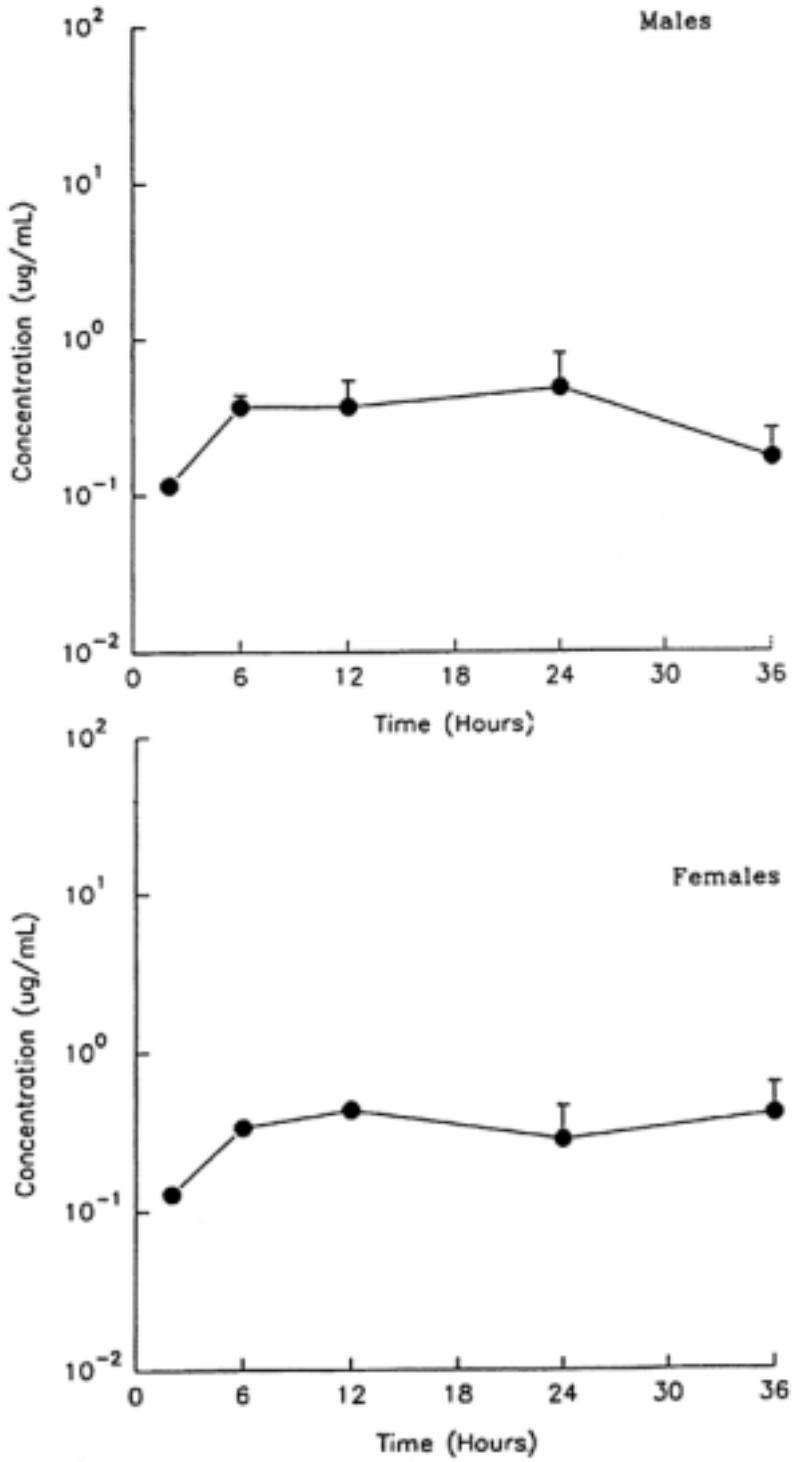

FIGURE I6
Plasma Concentrations of Anthraquinone in Aged Rats after a Single Gavage Dose of 100 mg/kg Anthraquinone

TABLE I7
Plasma Concentrations of Anthraquinone in Male Mice Administered Anthraquinone in Feed for 8 Days[a]

	833 ppm	2,500 ppm	7,500 ppm
n	3	3	3
Time of collection			
0800	0.138 ± 0.029	0.216 ± 0.044	0.419 ± 0.124
1000	0.066 ± 0.032	0.040 ± 0.027	0.614 ± 0.239
1200	0.077 ± 0.000[b]	0.039 ± 0.013	0.270 ± 0.012
1400	0.468 ± 0.257	0.088 ± 0.015	0.403 ± 0.130
1600	0.860 ± 0.329	0.114 ± 0.038	0.383 ± 0.040
1800	0.213 ± 0.015[b]	0.143 ± 0.012	0.629 ± 0.148
2000	0.218 ± 0.037	0.165 ± 0.010	0.607 ± 0.194
2200	0.149 ± 0.064	0.178 ± 0.048	0.535 ± 0.057
2400	0.157 ± 0.012	0.201 ± 0.025	0.473 ± 0.056
0200	0.154 ± 0.036[b]	0.185 ± 0.031	0.529 ± 0.060
0400	0.216 ± 0.108	0.133 ± 0.038	0.564 ± 0.145
0600	0.200[c]	0.234 ± 0.016	0.552 ± 0.052

[a] Data are given in μg/mL as mean ± standard error.
[b] n=2
[c] n=1; no standard error calculated

TABLE I8
Toxicokinetic Parameters in Male Mice Administered Anthraquinone in Feed for 8 Days[a]

Concentration (ppm)	C_{min} (μg/mL)	T_{min}	C_{max} (μg/mL)	T_{max}
833	0.066	1000	0.860	1600
2,500	0.039	1200	0.235	0600
7,500	0.270	1200	0.629	1800

[a] C_{min}=minimum mean concentration; T_{min}=time of minimum mean concentration; C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration

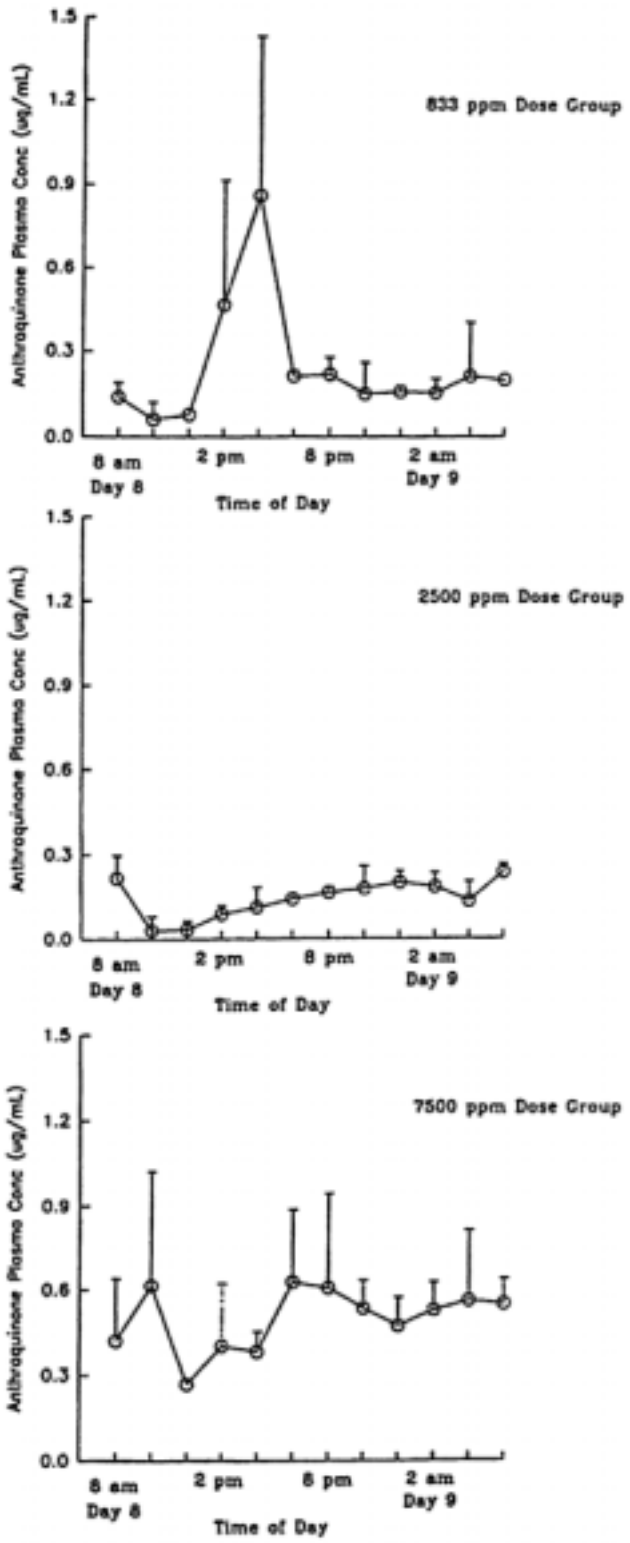

FIGURE I7
Plasma Concentrations of Anthraquinone in Male Mice Administered Anthraquinone in Feed for 8 Days

TABLE I9
Plasma Concentrations of Anthraquinone in Mice at the 12-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone[a]

	833 ppm	2,500 ppm	7,500 ppm
n	2	2	2
Male			
Time of collection			
0600	0.136 ± 0.011	0.156 ± 0.009	0.375 ± 0.024
1100	0.116 ± 0.003	0.094 ± 0.005	0.332 ± 0.019
1600	0.094 ± 0.020	0.085 ± 0.016	0.224 ± 0.038
2100	0.076 ± 0.024	0.104 ± 0.028	0.333 ± 0.053
0200	0.119[b]	0.143 ± 0.000	0.445 ± 0.016
Female			
Time of collection			
0600	0.153 ± 0.019	0.174 ± 0.052	0.465 ± 0.185
1100	0.102 ± 0.012	0.128 ± 0.006	0.311 ± 0.139
1600	0.082 ± 0.028	0.130 ± 0.015	0.296 ± 0.032
2100	0.082 ± 0.002	0.084 ± 0.015	0.215 ± 0.010
0200	0.092 ± 0.010	0.121 ± 0.010	0.314 ± 0.008

[a] Data are given in μg/mL as mean ± standard error.
[b] n=1; no standard error calculated

TABLE I10
Toxicokinetic Parameters in Mice at the 12-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone[a]

	Concentration (ppm)	C_{min} (μg/mL)	T_{min}	C_{max} (μg/mL)	T_{max}
Male					
	833	0.076	2100	0.136	0600
	2,500	0.085	1600	0.156	0600
	7,500	0.224	1600	0.446	0200
Female					
	833	0.082	1600 and 2100	0.153	0600
	2,500	0.084	2100	0.174	0600
	7,500	0.215	2100	0.465	0600

[a] C_{min}=minimum mean concentration; T_{min}=time of minimum mean concentration; C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration

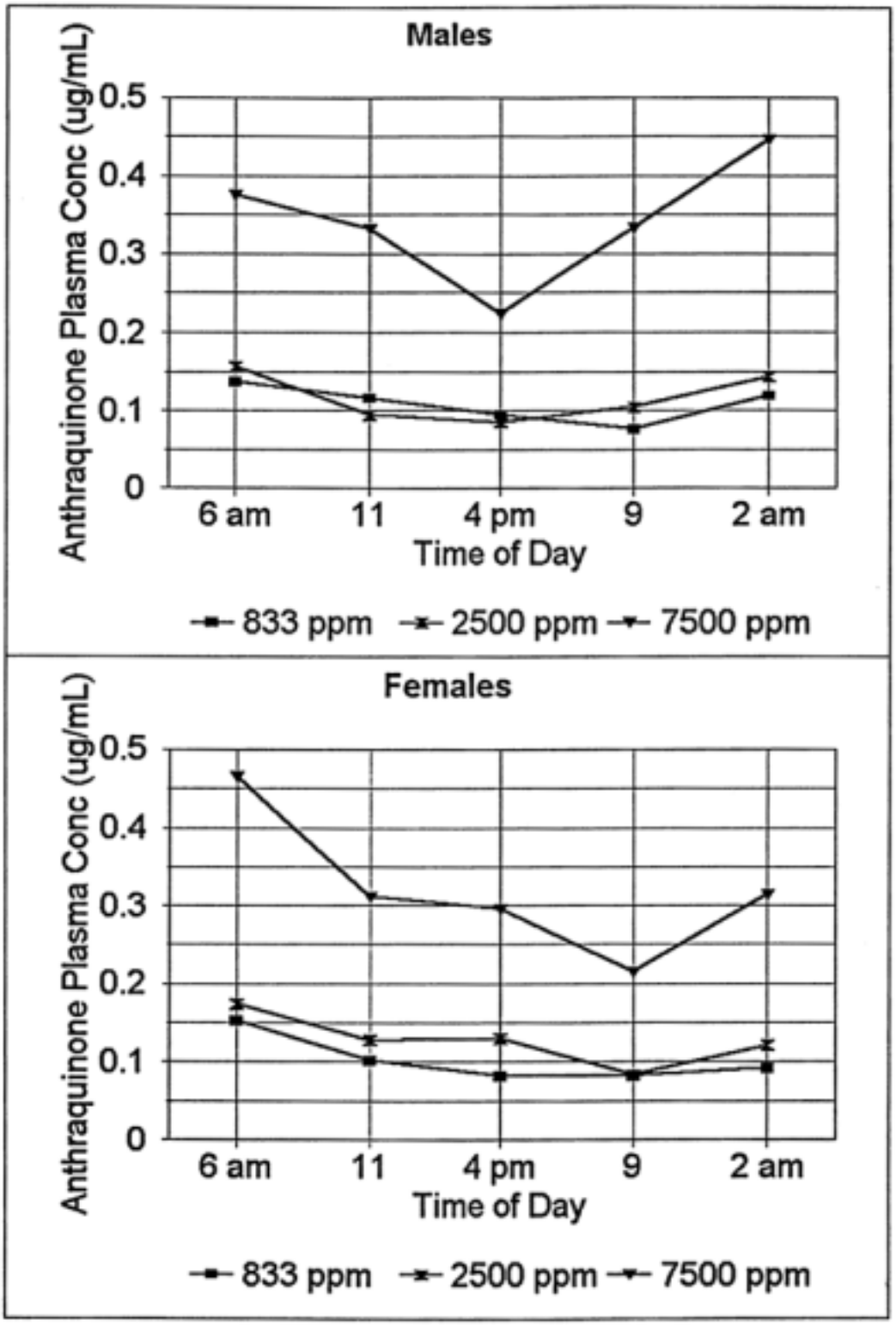

FIGURE I8
Plasma Concentrations of Anthraquinone in Mice at the 12-Month Interim Evaluation in the 2-Year Feed Study of Anthraquinone

TABLE I11
Plasma Concentrations of Anthraquinone in Aged Mice after a Single Gavage Dose of 200 mg/kg Anthraquinone[a]

Time after Dosing (hours)	Concentration[b] (μg/mL)
Male	
1	0.270 ± 0.061
2	0.353 ± 0.041
4	0.757 ± 0.049
8	1.050 ± 0.133
12	0.940 ± 0.060[c]
Female	
1	0.157 ± 0.009
2	0.607 ± 0.119
4	0.943 ± 0.073
8	0.880 ± 0.220[c]
12	0.465 ± 0.095[c]

[a] Three animals were bled at each time point.
[b] Data are given in μg/mL as the mean ± standard error.
[c] Two animals bled

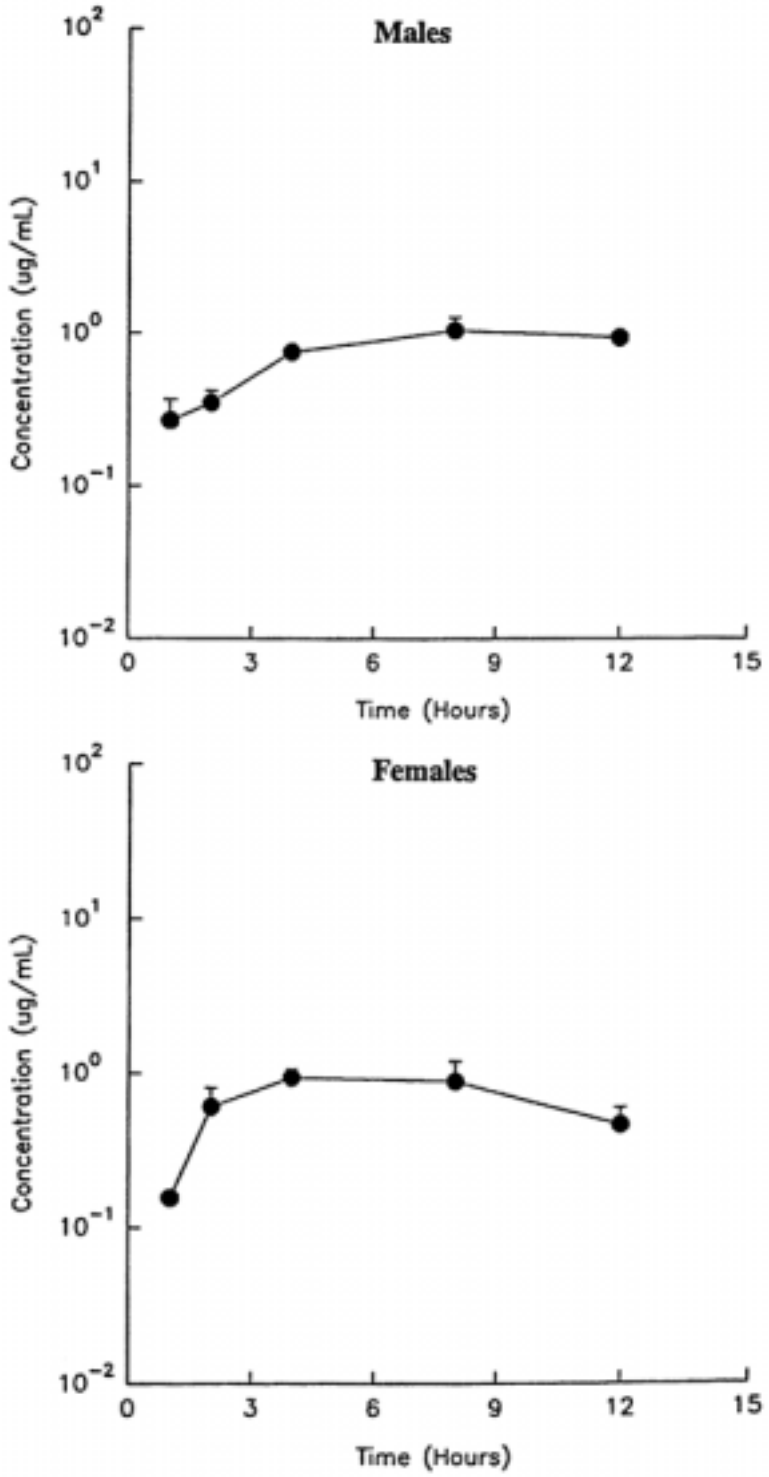

FIGURE I9
Plasma Concentrations of Anthraquinone in Aged Mice after a Single Gavage Dose of 200 mg/kg Anthraquinone

TABLE I12
Cardiac Output, Organ Volumes, and Organ Blood Perfusion Rates of Rats for the Physiologically Based Pharmacokinetic Model of Anthraquinone

	Male	Female
Cardiac Output (L/hr/kg$^{0.7}$)	14.7	14.7
Body Weight (kg)	0.287	0.170
Chyme Flow Rate (mL/hr)		
Stomach lumen	0.335	0.230
Intestine lumen	1.65	1.13
Colon lumen	2.08	1.43
Tissue Volumes (% of body weights)		
Arterial blood	0.466	0.43
Venous blood	1.362	1.29
Fat	7	7
Slowly perfused	54.2	56
Richly perfused	19.45	26.66
Kidney	1.48	0.85
Liver	3.7	4.5
Stomach	0.486	0.63
Stomach lumen	0.91	0.91
Intestine	1.58	2.05
Duodenum lumen	3.1	3.1
Jejunum lumen	3.1	3.1
Colon	0.795	1.03
Colon lumen	1.6	1.6
Tissue Capillary Volumes (% of tissue volume)		
Fat	2	2
Slowly perfused	2	2
Richly perfused	10	10
Kidney	16	16
Liver	13.8	13.8
Stomach	4.11	4.11
Intestine	2.65	2.65
Colon	2.33	2.33
Tissue Blood Flow (% of cardiac output)		
Fat	6.5	6.5
Slowly perfused	33.4	33.4
Richly perfused	27.4	27.4
Kidney	13.3	13.3
Liver (hepatic)	3.9	3.9
Stomach	1.2	1.2
Intestine	11.6	11.6
Colon	2.7	2.7

APPENDIX J
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF ANTHRAQUINONE J-2
PREPARATION AND ANALYSIS OF DOSE FORMULATIONS J-3
FIGURE J1 Infrared Absorption Spectrum of Anthraquinone J-4
FIGURE J2 Nuclear Magnetic Resonance Spectrum of Anthraquinone J-5
TABLE J1 Preparation and Storage of Dose Formulations in the Feed Studies of Anthraquinone . . J-6
TABLE J2 Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of Anthraquinone J-7
TABLE J3 Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Anthraquinone J-8

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF ANTHRAQUINONE

Anthraquinone was obtained from Zeneca Fine Chemicals (Wilmington, DE) in one lot (5893), which was used during the 14-week and 2-year studies. Identity, purity, and stability analyses were conducted by the study laboratory. **Analyses to identify and quantify impurities were conducted by the analytical chemistry laboratory, Battelle Columbus Operations (Columbus, OH).** Reports on analyses performed in support of the anthraquinone studies are on file at the National Institute of Environmental Health Sciences.

Upon receipt, the chemical, a golden yellow crystalline powder, was identified by infrared and proton nuclear magnetic resonance spectroscopy. Both spectra were consistent with the literature spectra (*Aldrich,* 1974, 1981) of anthraquinone. The infrared and nuclear magnetic spectra are presented in Figures J1 and J2.

The study laboratory determined the purity of lot 5893 by gas chromatography (Hewlett Packard, Palo Alto, CA). Gas chromatography was performed using a flame ionization detector **(GC/FID)** with a helium carrier gas at a flow rate of approximately 30 mL per minute. A DB-1 megabore capillary column (15 m × 0.53 mm) (J&W Scientific, Folsom, CA) was used with an oven temperature program of 100° to 270° C at 5° C per minute with a 5-minute hold at 270° C. **The analytical chemistry laboratory determined the purity of lot 5893 by reversed phase high-performance liquid chromatography with ultraviolet detection (HPLC/UV) (Spectra Physics LC, Mountain View, CA) and by GC/FID (Agilent Technologies, Palo Alto, CA). HPLC/UV was performed using an Inertsil ODS-2 column (150 mm × 3.0 mm), a mobile phase of acetonitrile:Milli-Q water (50:50, isocratic), a flow rate of 1.0 mL per minute, and ultraviolet detection at 250 nm. GC/FID was performed using an RTX-5 column (30 m × 0.25 mm, 0.25-μm film thickness; Restek, Bellefonte, PA), helium carrier gas at 1.5 mL per minute, and an oven temperature program of 110° to 280° C at 5° C per minute. HPLC/UV and GC/FID chromatograms were visually examined for the presence of an impurity at the same retention time as 9-nitroanthracene. The analytical chemistry laboratory identified the impurity by GC with a mass selective detector (GC/MS) (Agilent Technologies) using an RTX-5 column (30 m × 0.32 mm, 1.0-μm film thickness; Restek), helium carrier gas at 2 mL/minute, and an oven temperature program of 120° to 280° C at 5° C per minute followed by a 3-minute hold.**

Purity analysis by GC/FID indicated a purity of 99.9% with one impurity of 0.1%. Purity analysis by HPLC/UV showed a purity of 99.5% with two impurities of 0.3% and 0.2% relative to the anthraquinone peak. However, relative peak area measurements of purity by HPLC/UV may be less accurate because of likely differences in extinction coefficients between components. Therefore, an authentic standard was used to confirm (by GC/FID) that 9-nitroanthracene was present in the test article at 0.09% by standard addition. This compound was also confirmed by GC/MS as the greater of the two impurities seen by HPLC/UV. The lesser impurity detected by HPLC/UV was not identified, but was determined not to be 1- or 2-nitroanthracene using authentic standards.

The study laboratory conducted accelerated bulk stability studies on lot 5893 with gas chromatography using the system described for the purity analysis but with an isothermal oven temperature of 150° C for 10 minutes, 150° to 270° C at 20° C per minute, then a 10-minute hold at 270° C with octanophenone added as the internal standard. These studies indicated that anthraquinone is stable as a bulk chemical for up to 2 weeks when stored in sealed containers protected from ultraviolet light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in amber glass bottles with Teflon-lined caps for the 14-week and 2-year studies. Stability was monitored during the studies using gas chromatography. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared every 4 weeks by mixing anthraquinone with feed (Table J1). A premix of anthraquinone and feed was prepared by hand, then blended with feed in a Patterson-Kelly twin-shell blender for approximately 15 minutes, using an intensifier bar for the initial 5 minutes. Formulations were stored in polyethylene bags in sealed polypropylene buckets at room temperature for up to 35 days.

Homogeneity studies of the 1,875 and 30,000 ppm dose formulations and stability studies of a 230 ppm dose formulation were performed by the study laboratory using gas chromatography with the system described for the bulk stability studies. Homogeneity was confirmed. Stability was confirmed for 35 days for dose formulations stored at room temperature in sealed containers protected from light and for 7 days when stored at room temperature, exposed to air and light.

Periodic analyses of the dose formulations of anthraquinone were conducted by the study laboratory using gas chromatography with the system described for the bulk stability studies. During the 14-week studies, dose formulations from the beginning and end of the studies were analyzed (Table J2). Dose formulations for the 2-year studies were analyzed approximately every 8 or 12 weeks (Table J3). All dose formulations prepared for the 14-week studies (10/10) and 2-year studies (rats, 84/84; mice, 33/33) were within 10% of the target concentrations. For the 14-week studies, 80% (4/5) of animal room samples for rats and 90% (9/10) for mice were within 10% of the target concentrations. All animal room samples for rats (27/27) and 67% (8/12) for mice in the 2-year studies were within 10% of the target concentrations. Four animal room samples for the mice in the 2-year studies ranged from 13% to 19% less than the target concentrations; this was likely due to contamination of these samples with urine, feces, and bedding.

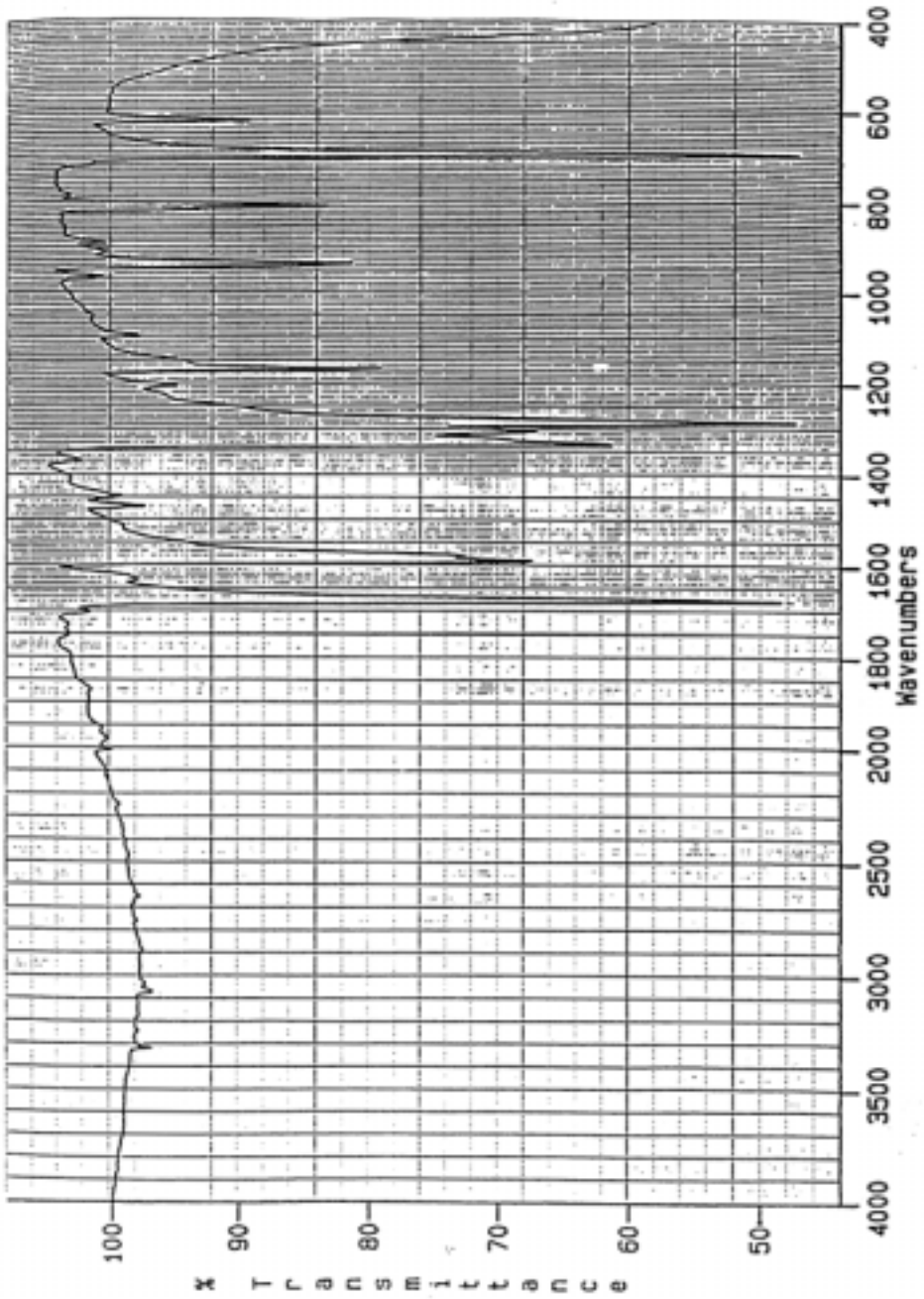

FIGURE J1
Infrared Absorption Spectrum of Anthraquinone

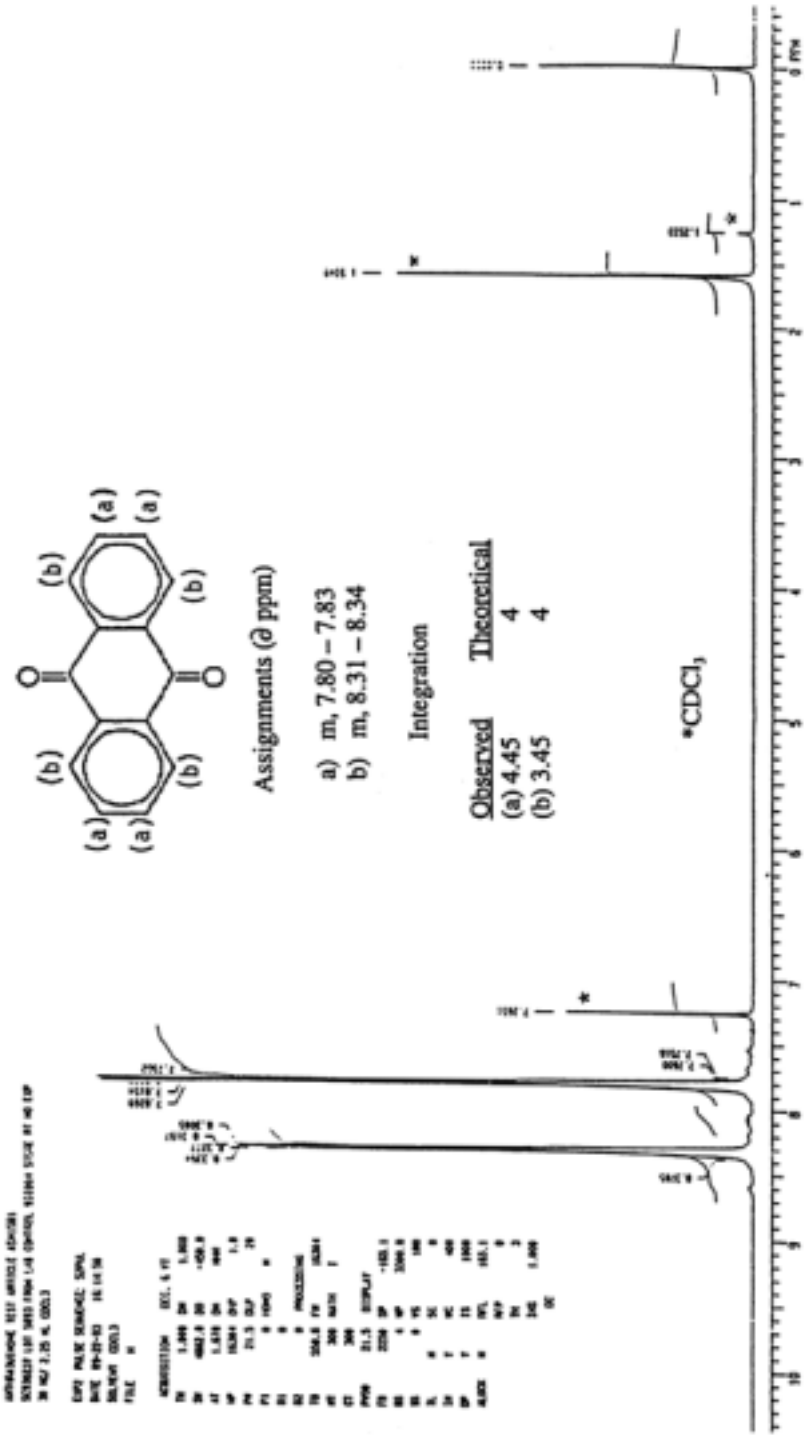

FIGURE J2
Nuclear Magnetic Resonance Spectrum of Anthraquinone

TABLE J1
Preparation and Storage of Dose Formulations in the Feed Studies of Anthraquinone

14-Week Studies	2-Year Studies
Preparation	
A premix of feed and anthraquinone was prepared, then layered into the remaining feed and blended in a Patterson-Kelly twin-shell blender with the intensifier bar on for 5 minutes and off for approximately 10 minutes. Doses were prepared every 4 weeks.	Same as 14-week studies; the premixes of the 469, 833, and 938 ppm formulations were ground with a Wiley laboratory mill equipped with a 1-mm sieve and then further mixed with undosed feed before being layered into the blender.
Chemical Lot Number	
5893	5893
Maximum Storage Time	
35 days	35 days
Storage Conditions	
Stored in polyethylene bags inside sealed polypropylene buckets at room temperature	Same as 14-week studies
Study Laboratory	
Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)

TABLE J2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of Anthraquinone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
January 10, 1994	January 12-13, 1994	1,875	1,878	0
		3,750	3,770	+1
		7,500	7,916	+6
		15,000	14,859	–1
		30,000	29,338	–2
	February 22-24, 1994[b]	1,875	1,925	+3
		3,750	3,899	+4
		7,500	8,393	+12
		15,000	15,620	+4
		30,000	31,517	+5
March 7, 1994	March 8-9, 1994	1,875	1,863	–1
		3,750	3,749	0
		7,500	7,373	–2
		15,000	14,618	–3
		30,000	30,877	+3
Mice				
January 10, 1994	January 12-13, 1994	1,875	1,878	0
		3,750	3,770	+1
		7,500	7,916	+6
		15,000	14,859	–1
		30,000	29,338	–2
	February 22-24, 1994[c]	1,875	1,765	–6
		3,750	3,697	–1
		7,500	6,378	–15
		15,000	14,656	–2
		30,000	27,924	–7
	February 22-24, 1994[d]	1,875	1,732	–8
		3,750	3,741	0
		7,500	6,723	–10
		15,000	14,260	–5
		30,000	29,864	0
March 7, 1994	March 8-9, 1994	1,875	1,863	–1
		3,750	3,749	0
		7,500	7,373	–2
		15,000	14,618	–3
		30,000	30,877	+3

[a] Results of duplicate analyses
[b] Animal room samples for rats
[c] Animal room samples for male mice
[d] Animal room samples for female mice

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Anthraquinone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
October 24, 1994	October 25, 1994	469	469	0
		938	951	+1
		1,875	1,876	0
		3,750	3,775	+1
	December 8-9, 1994[b]	469	458	–2
		938	986	+5
		1,875	1,888	+1
		3,750	3,869	+3
December 19, 1994	December 19-20, 1994	469	464	–1
		469	467	0
		938	959	+2
		938	964	+3
		1,875	1,856	–1
		1,875	1,873	0
		3,750	3,927	+5
		3,750	3,876	+3
March 13, 1995	March 14-15, 1995	469	456	–3
		469	467	0
		938	922	–2
		938	958	+2
		1,875	1,925	+3
		1,875	1,894	+1
		3,750	3,887	+4
		3,750	3,788	+1
June 5, 1995	June 8-9, 1995	469	494	+5
		469	488	+4
		938	983	+5
		938	985	+5
		1,875	1,906	+2
		1,875	1,939	+3
		3,750	4,039	+8
		3,750	3,827	+2
	July 10-11, 1995[b]	469	455	–3
		469	454	–3
		938	910	–3
		938	946	+1
		1,875	1,951	+4
		3,750	3,566	–5
		3,750	3,720	–1
July 31, 1995	August 1-2, 1995	469	485	+3
		469	483	+3
		938	959	+2
		938	942	0
		1,875	1,908	+2
		1,875	1,887	+1
		3,750	3,952	+5
		3,750	3,810	+2

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Anthraquinone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
October 23, 1995	October 26-27, 1995	469	465	−1
		469	469	0
		938	915	−2
		938	944	+1
		1,875	1,861	−1
		1,875	1,923	+3
		3,750	3,851	+3
		3,750	3,861	+3
January 15, 1996	January 16-17, 1996	469	471	0
		469	495	+6
		938	936	0
		938	944	+1
		1,875	1,860	−1
		1,875	1,956	+4
		3,750	3,926	+5
		3,750	3,955	+5
	February 27-28, 1996[b]	469	432	−8
		469	464	−1
		938	997	+6
		938	977	+4
		1,875	1,890	+1
		1,875	1,892	+1
		3,750	3,705	−1
		3,750	3,592	−4
March 11, 1996	March 14-15, 1996	469	451	−4
		469	472	+1
		938	879	−6
		938	904	−4
		1,875	1,874	0
		1,875	1,869	0
		3,750	3,822	+2
		3,750	3,684	−2
June 3, 1996	June 4-5, 1996	469	454	−3
		469	480	+2
		938	925	−1
		938	961	+2
		1,875	1,839	−2
		1,875	1,902	+1
		3,750	3,821	+2
		3,750	3,806	+1

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Anthraquinone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
August 26, 1996	August 28-29, 1996	469	465	–1
		469	430	–8
		938	931	–1
		938	887	–5
		1,875	1,898	+1
		1,875	1,911	+2
		3,750	3,972	+6
		3,750	3,913	+4
	October 1-2, 1996[b]	469	428	–9
		469	444	–5
		938	854	–9
		938	928	–1
		1,875	1,872	0
		1,875	1,860	–1
		3,750	3,781	+1
		3,750	3,798	+1
October 21, 1996	October 22-24, 1996	469	464	–1
		469	448	–4
		938	931	–1
		938	941	0
		1,875	1,903	+1
		1,875	1,869	0
		3,750	3,855	+3
		3,750	3,796	+1
Mice				
October 24, 1994	October 25, 1994	833	828	–1
		2,500	2,522	+1
		7,500	7,297	–3
	December 8-9, 1994[b]	833	721	–13
		2,500	2,033	–19
		7,500	6,195	–17
December 19, 1994	December 19-20, 1994	833	792	–5
		2,500	2,483	–1
		7,500	7,783	+4
March 13, 1995	March 14-15, 1995	833	825	–1
		2,500	2,540	+2
		7,500	7,649	+2
June 5, 1995	June 8-9, 1995	833	885	+6
		2,500	2,440	–2
		7,500	7,727	+3
	July 10-11, 1995[b]	833	792	–5
		2,500	2,561	+2
		7,500	7,863	+5

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Anthraquinone

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
July 31, 1995	August 1-2, 1995	833	824	−1
		2,500	2,536	+1
		7,500	7,706	+3
October 23, 1995	October 26-27, 1995	833	810	−3
		2,500	2,509	0
		7,500	7,637	+2
January 15, 1996	January 16-17, 1996	833	804	−3
		2,500	2,417	−3
		7,500	8,124	+8
	February 27-28, 1996[b]	833	765	−8
		2,500	2,434	−3
		7,500	7,230	−4
March 11, 1996	March 14-15, 1996	833	829	0
		2,500	2,507	0
		7,500	7,609	+1
June 3, 1996	June 4-5, 1996	833	779	−6
		2,500	2,470	−1
		7,500	7,626	+2
August 26, 1996	August 28-29, 1996	833	846	+2
		2,500	2,539	+2
		7,500	7,837	+4
	October 1-2, 1996[b]	833	800	−4
		2,500	2,251	−10
		7,500	6,237	−17
October 21, 1996	October 22-24, 1996	833	794	−5
		2,500	2,483	−1
		7,500	7,386	−2

[a] Results of duplicate analyses
[b] Animal room samples

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

TABLE K1 Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Anthraquinone K-2
TABLE K2 Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Anthraquinone K-4
TABLE K3 Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of Anthraquinone K-6
TABLE K4 Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Anthraquinone K-7

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

	0 ppm		469 ppm			938 ppm		
Week	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)
1	14.6	113	14.4	112	60	14.5	114	124
2	15.5	149	15.3	148	48	14.7	149	97
6	17.4	252	16.8	246	32	16.5	241	67
10	16.9	301	16.5	294	26	16.9	293	57
14	18.6	336	18.1	329	26	17.6	323	53
18	17.5	361	17.2	354	23	16.6	346	47
22	18.3	379	17.5	373	22	17.3	367	46
26	16.1	395	16.5	393	20	16.2	389	41
30	17.9	409	17.7	407	20	17.6	404	43
34	18.2	416	17.5	419	20	16.8	414	40
38	17.1	423	16.9	425	19	16.7	418	39
42	17.9	430	17.4	432	19	18.3	423	42
46	18.2	436	17.9	440	19	18.3	433	42
50	17.5	441	17.2	439	18	17.8	434	40
54	18.2	447	17.4	443	18	17.6	438	40
58	16.9	458	16.6	450	17	16.5	444	37
62	18.0	466	17.8	461	18	17.9	453	39
66	17.0	468	17.3	461	18	17.5	457	38
70	16.6	468	16.6	461	17	15.9	453	35
74	15.6	471	17.1	456	18	16.5	455	36
78	15.8	475	16.9	458	17	16.4	449	36
82	15.8	476	16.3	456	17	16.5	447	36
86	14.9	471	14.7	444	16	15.7	439	35
90	16.8	462	16.8	442	18	17.5	432	40
94	14.0	448	15.7	425	17	16.7	427	38
98	16.1	450	17.0	429	19	16.1	425	37
102	14.8	430	15.2	405	18	15.4	400	38
Mean for weeks								
1-13	16.1	204	15.8	200	42	15.6	199	86
14-52	17.7	403	17.4	401	21	17.3	395	43
53-102	16.2	461	16.6	446	17	16.6	440	37

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

	1,875 ppm			3,750 ppm		
Week	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
1	14.3	113	238	13.3	113	442
2	14.8	148	188	14.3	145	370
6	16.5	242	128	16.0	237	254
10	17.0	286	111	16.7	285	220
14	17.6	317	104	17.7	322	206
18	16.9	349	91	17.0	348	183
22	17.3	370	88	17.6	366	180
26	17.2	390	83	16.9	383	166
30	17.7	405	82	18.4	400	173
34	17.4	414	79	17.5	409	161
38	17.2	420	77	17.6	417	159
42	17.7	429	77	18.1	426	159
46	18.5	440	79	18.7	431	163
50	18.2	437	78	17.1	429	149
54	17.7	440	76	18.4	425	162
58	18.0	446	76	16.2	430	141
62	18.9	457	77	17.7	444	149
66	18.1	464	73	18.0	447	151
70	16.5	459	67	16.4	443	139
74	17.0	459	69	17.0	449	142
78	16.9	456	69	16.3	442	139
82	16.1	452	67	15.9	438	136
86	14.8	441	63	16.1	434	139
90	17.6	440	75	16.6	430	145
94	16.3	423	72	16.8	420	150
98	17.0	418	76	16.9	414	153
102	17.7	414	80	16.4	402	153
Mean for weeks						
1-13	15.6	197	166	15.1	195	322
14-52	17.6	397	84	17.7	393	170
53-102	17.1	444	72	16.8	432	146

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

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

	0 ppm		469 ppm			938 ppm		
Week	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)
1	11.3	101	11.7	101	54	10.9	102	105
2	11.3	118	11.5	119	45	10.7	117	90
6	10.8	165	10.9	161	32	11.1	156	70
10	8.9	182	9.4	176	25	9.9	171	57
14	11.1	195	11.0	187	28	10.2	182	55
18	10.3	208	10.7	199	25	9.1	187	48
22	9.8	215	9.1	202	21	9.8	195	49
26	9.7	226	9.5	210	21	9.6	203	46
30	10.6	231	9.4	213	21	9.8	206	47
34	9.9	238	10.1	218	22	9.4	209	44
38	10.5	242	8.9	213	19	10.2	209	48
42	10.3	250	10.3	226	21	9.7	214	45
46	10.3	261	10.7	232	22	9.9	219	44
50	9.8	261	10.3	233	21	10.0	219	45
54	11.9	269	10.7	239	21	11.2	222	50
58	11.3	271	10.5	247	20	10.7	230	46
62	12.2	293	12.1	261	22	11.4	243	46
66	11.8	302	11.3	267	20	10.9	252	43
70	11.2	311	11.5	273	20	11.1	258	42
74	11.4	318	11.3	281	19	12.4	270	45
78	10.8	320	11.3	285	19	10.8	272	39
82	10.9	322	11.9	290	19	10.7	275	38
86	11.6	330	11.2	290	18	11.0	280	39
90	11.8	335	11.9	299	19	11.4	283	40
94	11.7	338	12.6	299	20	11.4	281	40
98	12.0	345	12.0	306	18	12.2	296	41
102	12.0	343	11.6	305	18	10.9	290	37
Mean for weeks								
1-13	10.6	141	10.9	139	39	10.7	137	80
14-52	10.2	233	10.0	213	22	9.8	204	47
53-102	11.6	315	11.5	280	19	11.2	265	42

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

	1,875 ppm			3,750 ppm		
Week	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
1	10.4	100	194	10.2	102	375
2	10.5	116	169	11.0	117	352
6	10.9	156	132	10.9	157	260
10	10.3	169	114	9.1	166	207
14	10.6	185	107	10.6	177	226
18	9.2	191	90	10.2	184	208
22	8.8	192	86	9.8	191	193
26	9.2	199	87	9.4	195	181
30	9.7	203	90	9.9	197	189
34	9.4	205	86	10.1	202	189
38	9.7	205	88	9.5	199	179
42	10.0	210	90	10.0	204	184
46	10.2	213	89	10.4	209	188
50	10.1	214	89	10.6	211	188
54	10.7	219	91	10.6	214	185
58	10.7	225	89	11.0	222	186
62	10.9	235	87	11.5	234	185
66	11.5	245	88	11.0	238	173
70	10.6	251	80	11.0	249	166
74	12.0	262	86	11.3	258	164
78	11.1	267	78	11.6	262	166
82	10.5	273	72	11.1	264	158
86	11.2	277	76	11.5	268	161
90	11.4	281	76	11.6	275	157
94	10.1	274	69	11.5	271	159
98	12.7	288	83	12.0	277	163
102	11.1	284	73	10.9	272	150
Mean for weeks						
1-13	10.5	135	152	10.3	135	298
14-52	9.7	202	90	10.1	197	192
53-102	11.1	260	81	11.3	254	167

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

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

	0 ppm		833 ppm			2,500 ppm			7,500 ppm		
Week	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
2	4.4	23.0	4.7	23.2	167	4.8	23.2	517	4.4	23.4	1,426
6	4.6	28.1	5.1	28.7	148	5.2	28.6	456	5.1	28.4	1,334
10	4.8	32.1	4.6	33.3	116	4.8	33.4	358	4.7	32.6	1,080
14	4.5	35.5	4.6	37.0	103	4.8	36.6	326	4.6	35.8	954
18	4.5	38.7	4.4	40.5	90	4.5	40.1	280	4.5	39.4	853
22	3.9	41.6	4.1	43.3	79	4.1	42.6	239	4.5	41.8	803
26	4.2	43.3	4.3	44.6	80	4.2	43.8	242	4.2	42.9	743
30	4.1	45.4	4.3	46.1	77	4.5	45.9	245	4.4	45.0	730
34	4.2	46.4	4.2	47.1	75	4.2	47.2	225	4.2	46.1	689
38	4.4	47.3	4.3	47.6	74	4.4	47.4	230	4.3	46.8	693
42	4.3	48.2	4.5	48.5	77	4.3	48.1	226	4.5	47.9	697
46	4.4	48.1	4.4	48.3	76	4.3	48.2	225	4.4	47.9	686
50	4.5	49.4	4.7	50.3	77	4.5	50.3	223	4.5	49.6	686
54	4.4	48.4	4.5	49.5	76	4.5	50.0	224	4.5	49.0	696
58	4.6	49.1	4.6	49.9	77	4.5	50.5	222	4.6	49.8	694
61	4.7	50.1	4.6	50.6	76	4.6	50.1	227	4.7	50.5	699
66	4.1	49.4	4.4	49.9	73	4.2	50.3	211	4.3	50.8	630
70	4.5	49.6	4.6	50.2	77	4.5	50.5	224	4.6	49.7	696
74	4.5	49.4	4.6	50.9	76	4.5	50.3	225	4.5	50.4	675
78	4.6	49.3	4.5	50.7	74	4.5	50.2	222	4.5	48.6	688
82	4.8	49.1	4.8	50.2	79	4.7	49.6	234	4.6	46.8	743
86	4.8	49.0	4.6	50.0	77	4.7	49.7	237	4.7	46.2	768
90	5.0	48.5	4.8	49.8	81	4.8	49.0	243	4.7	44.0	797
94	4.7	48.6	4.5	48.2	77	4.8	48.3	246	4.8	41.7	857
98	4.9	48.4	5.1	48.1	88	5.0	47.6	264	5.3	40.0	992
102	4.6	47.8	4.6	46.3	83	4.6	46.0	252	5.0	37.9	989
Mean for weeks											
1-13	4.6	27.8	4.8	28.4	144	4.9	28.4	444	4.7	28.1	1,280
14-52	4.3	44.4	4.4	45.3	81	4.4	45.0	246	4.4	44.3	753
53-102	4.6	49.0	4.6	49.6	78	4.6	49.4	233	4.7	46.6	763

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

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

	0 ppm		833 ppm			2,500 ppm			7,500 ppm		
Week	Feed (g)[a]	Body Weight (g)	Feed (g)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g)	Body Weight (g)	Dose (mg/kg)	Feed (g)	Body Weight (g)	Dose (mg/kg)
1	3.9	17.0	3.8	17.0	186	4.2	17.0	616	4.6	17.4	2,001
2	2.7	18.4	3.0	17.5	145	3.3	18.6	443	3.6	18.9	1,445
6	4.7	22.9	4.8	22.3	179	4.4	23.1	472	4.7	23.4	1,509
10	5.1	26.5	4.1	26.5	128	4.2	26.3	398	4.5	27.7	1,229
14	4.2	29.5	3.7	29.4	105	4.3	30.2	357	4.1	30.6	1,002
18	3.8	33.7	3.8	33.6	95	3.9	34.3	285	4.1	34.6	893
22	3.9	36.4	3.8	36.6	86	3.9	36.5	266	4.3	36.7	873
26	3.9	39.0	3.8	38.4	82	3.9	38.4	256	4.4	37.7	866
30	4.6	41.4	4.0	40.8	82	4.1	41.3	249	4.0	39.7	749
34	3.5	43.8	3.5	43.6	66	3.4	43.4	198	3.7	42.8	658
38	3.7	45.4	3.5	45.1	65	3.5	45.0	194	3.7	44.0	631
42	3.7	47.2	3.7	47.0	65	3.4	46.6	184	3.6	45.4	599
46	3.8	48.5	4.3	48.2	75	3.8	47.7	199	4.0	47.1	631
50	3.6	50.8	3.7	50.2	61	3.6	49.7	183	3.7	48.4	581
54	3.5	51.3	3.5	51.4	56	3.3	50.8	164	3.5	49.7	526
58	3.6	52.1	3.7	50.7	61	3.4	51.1	168	3.9	49.6	592
61	3.7	53.3	3.8	53.1	59	3.8	53.0	180	3.8	50.8	566
66	3.3	53.5	3.5	54.0	55	3.6	54.3	164	3.4	50.6	511
70	3.4	54.5	3.6	54.2	55	3.5	55.1	160	3.7	52.9	520
74	3.6	55.9	3.7	55.3	56	3.7	56.2	162	3.7	54.4	516
78	3.4	56.1	3.4	56.0	51	3.5	56.5	156	3.5	53.6	485
82	3.7	56.0	3.8	56.0	56	3.7	56.9	162	3.8	54.6	518
86	3.6	56.1	3.7	55.8	55	3.5	55.3	160	3.8	55.3	514
90	3.5	56.5	4.0	56.8	59	3.7	55.8	167	3.7	54.5	512
94	3.9	55.8	3.8	56.6	56	4.0	56.5	178	3.9	54.1	538
98	4.1	56.8	4.2	56.3	62	4.3	54.2	200	4.5	53.2	628
102	3.6	54.8	3.8	55.2	58	3.8	53.2	178	4.0	51.3	578
Mean for weeks											
1-13	4.1	21.2	3.9	20.8	159	4.0	21.3	482	4.4	21.8	1,546
14-52	3.9	41.6	3.8	41.3	78	3.8	41.3	237	4.0	40.7	748
53-102	3.6	54.8	3.7	54.7	57	3.7	54.5	169	3.8	52.7	539

[a] Grams of feed consumed per animal per day
[b] Milligrams of anthraquinone 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 L-2
TABLE L2 Vitamins and Minerals in NIH-07 Rat and Mouse Ration L-2
TABLE L3 Nutrient Composition of NIH-07 Rat and Mouse Ration L-3
TABLE L4 Contaminant Levels in NIH-07 Rat and Mouse Ration L-4

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)	22.76 ± 0.72	20.7 – 24.2	24
Crude fat (% by weight)	5.21 ± 0.29	4.60 – 5.70	24
Crude fiber (% by weight)	3.39 ± 0.29	2.80 – 4.00	24
Ash (% by weight)	6.41 ± 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)	6,468 ± 855	4,440 – 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)	18.80 ± 3.72	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)	0.50 ± 0.21	0.10 – 0.80	24
Cadmium (ppm)	0.06 ± 0.03	0.04 – 0.15	24
Lead (ppm)	0.23 ± 0.11	0.10 – 0.50	24
Mercury (ppm)	<0.02		24
Selenium (ppm)	0.31 ± 0.08	0.10 – 0.42	24
Aflatoxins (ppm)	<5.0		24
Nitrate nitrogen (ppm)[c]	8.41 ± 4.80	0.80 – 19.3	24
Nitrite nitrogen (ppm)[c]	1.20 ± 1.19	0.04 – 4.80	24
BHA (ppm)[d]	1.09 ± 1.10	0.01 – 5.00	24
BHT (ppm)[d]	1.30 ± 1.10	0.10 – 5.00	24
Aerobic plate count (CFU/g)	244,042 ± 289,839	43,000 – 1,200,000	24
Coliform (MPN/g)	575 ± 1,107	3 – 4,300	24
Escherichia coli (MPN/g)	<10		24
Salmonella (MPN/g)	Negative		24
Total nitrosoamines (ppb)[e]	11.03 ± 3.87	3.2 – 19.7	24
N-Nitrosodimethylamine (ppb)[e]	9.14 ± 3.96	1.0 – 18.00	24
N-Nitrosopyrrolidine (ppb)[e]	1.89 ± 0.71	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	0.12 ± 0.18	0.02 – 0.91	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfane 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 M-2
RESULTS M-4

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	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	4 weeks, study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	4 weeks, study termination
Sendai	4 weeks, study termination
Immunofluorescence Assay	
PVM	4 weeks
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	4 weeks, study termination
KRV (Kilham rat virus)	4 weeks, study termination
2-Year Study	
ELISA	
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	4 weeks, 6, 12, and 18 months, study termination
RCV/SDA	4 weeks, 6, 12, and 18 months, study termination
Sendai	4 weeks, 6, 12, and 18 months, study termination
Hemagglutination Inhibition	
H-1	4 weeks, 6, 12, and 18 months, study termination
KRV	4 weeks, 6, 12, and 18 months, study termination

Method and Test	Time of Analysis
MICE	
14-Week Study	
ELISA	
Ectromelia virus	4 weeks, study termination
EDIM (epizootic diarrhea of infant mice)	4 weeks, study termination
GDVII (mouse encephalomyelitis virus)	4 weeks, study termination
LCM (lymphocytic choriomeningitis virus)	4 weeks, study termination
Mouse adenoma virus-FL	4 weeks, study termination
MHV (mouse hepatitis virus)	4 weeks, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	4 weeks, study termination
Reovirus 3	4 weeks, study termination
Sendai	4 weeks, study termination
Immunofluorescence Assay	
EDIM	Study termination
Hemagglutination Inhibition	
K (papovavirus)	4 weeks, study termination
MVM (minute virus of mice)	4 weeks, study termination
Polyoma virus	4 weeks, study termination
2-Year Study	
ELISA	
Ectromelia virus	4 weeks, 6, 12, and 18 months, study termination
EDIM	4 weeks, 6, 12, and 18 months, study termination
GDVII	4 weeks, 6, 12, and 18 months, study termination
LCM	4 weeks, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	4 weeks, 6, 12, and 18 months, study termination
MHV	4 weeks, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	4 weeks, 6, 12, and 18 months, study termination
Reovirus 3	4 weeks, 6, 12, and 18 months, study termination
Sendai	4 weeks, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Ectromelia virus	6 and 18 months
LCM	6, 12, and 18 months
MCMV (mouse cytomegalovirus)	Study termination
MHV	6 months
Sendai	12 months
Hemagglutination Inhibition	
K	4 weeks, 6, 12, and 18 months, study termination
MVM	4 weeks, 6, 12, and 18 months, study termination
Polyoma virus	4 weeks, 6, 12, and 18 months, study termination

RESULTS

Four rats had positive titers for *M. arthritidis* at the end of the 2-year study. 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 sporadic 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 false positives.

APPENDIX N
SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F$_1$ MICE

INTRODUCTION N-2
MATERIALS AND METHODS N-2
RESULTS N-3
REFERENCES N-4
TABLE N1 Plasma Concentrations of Anthraquinone in F344/N Rats after a Single Intravenous Injection of 2 mg/kg Anthraquinone N-5
FIGURE N1 Plasma Concentrations of Anthraquinone in F344/N Rats after a Single Intravenous Injection of 2 mg/kg Anthraquinone N-6
TABLE N2 Plasma Concentrations of Anthraquinone in F344/N Rats after a Single Gavage Dose of Anthraquinone N-7
FIGURE N2 Plasma Concentrations of Anthraquinone in F344/N Rats after a Single Gavage Dose of Anthraquinone N-8
FIGURE N3 Dose Versus AUC for F344/N Rats after a Single Gavage Dose of Anthraquinone N-9
TABLE N3 Plasma Concentrations of Anthraquinone in B6C3F$_1$ Mice after a Single Intravenous Injection of 4 mg/kg Anthraquinone N-10
FIGURE N4 Plasma Concentrations of Anthraquinone in B6C3F$_1$ Mice after a Single Intravenous Injection of 4 mg/kg Anthraquinone N-11
TABLE N4 Plasma Concentrations of Anthraquinone in B6C3F$_1$ Mice after a Single Gavage Dose of Anthraquinone N-12
FIGURE N5 Plasma Concentrations of Anthraquinone in B6C3F$_1$ Mice after a Single Gavage Dose of Anthraquinone N-13
FIGURE N6 Dose Versus AUC for B6C3F$_1$ Mice after a Single Gavage Dose of Anthraquinone N-14
TABLE N5 Summary of Toxicokinetic Data from a Single Dose Intravenous and Oral Gavage Anthraquinone Study in F344/N Rats N-15
TABLE N6 Summary of Toxicokinetic Data from a Single Dose Intravenous and Oral Gavage Anthraquinone Study in B6C3F$_1$ Mice N-16

SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F$_1$ MICE

INTRODUCTION

Anthraquinone is used as an intermediate in the manufacture of dyes and pigments as well as numerous other organic compounds. It has been used in the pulp and paper industry as an additive in the Kraft pulping process (Voss, 1981). Anthraquinone has also been used as a catalyst in the isomerization of vegetable oils, an accelerator in nickel electroplating, and as a bird repellant that was sprayed on growing crops or applied as a seed dressing (Meister, 1987). Single-dose intravenous and oral gavage toxicokinetic studies of anthraquinone in male and female F344/N rats and B6C3F$_1$ mice were conducted by Battelle Columbus Laboratories (Columbus, OH).

MATERIALS AND METHODS

Anthraquinone was obtained from Zeneca Fine Chemicals (Wilmington, DE) in one lot (5893), which was also used in the 2-year studies conducted at Battelle Columbus Laboratories. Results of identity, purity, and stability analyses of lot 5893 are presented in Appendix J. Dose formulations for gavage administration were prepared in 0.2% aqueous methylcellulose and 0.1% Tween 80. Dose formulations for intravenous injection were prepared by mixing anthraquinone with dimethylsulfoxide. Chloroform extracts of the dose formulations were analyzed by the study laboratory using gas chromatography methods similar to those described for the accelerated bulk stability studies in Appendix J.

Male and female F344/N rats were obtained from Hilltop Lab Animals, Inc. (Scottdale, PA); male and female B6C3F$_1$ mice were obtained from Charles River Laboratories (Portage, MI). Rats were acclimated for 6 (rats with femoral vein catheter implants) or 10 days and mice were acclimated for 10 or 12 days prior to being assigned to the study. Rats and mice were housed individually in polycarbonate cages containing hardwood bedding (Sani-Chips®, P.J. Murphy Forest Products Corp., Montville, NJ). Room environmental conditions included a temperature range of 69° to 75° F, relative humidity of 35% to 65%, 12:12 hour light/dark cycle, and a minimum of 10 fresh air changes per hour. Animals received NIH-07 open formula diet and water *ad libitum*.

Groups of 14 male and 14 female rats were administered a single intravenous injection of 2 mg anthraquinone/kg body weight; groups of 12 male and 12 female rats were administered a single gavage dose of 40, 100, or 400 mg/kg. The dosing volume was 2 mL/kg by intravenous injection or 5 mL/kg by gavage. Groups of 27 male and 27 female mice were administered a single intravenous injection of 4 mg/kg; groups of 24 male and 24 female mice were administered a single dose of 80, 200, or 800 mg/kg by gavage. The dosing volume was 4 mL/kg by intravenous injection or 10 mL/kg by gavage. The animals were anesthetized with a mixture of carbon dioxide and oxygen, and blood samples were collected by retroorbital (rats) or cardiac (mice) puncture from three male and three female animals per time point.

In the rat intravenous injection study, samples were collected at 2, 10, 20, 40, 60, 120, 300, 480, and 600 minutes after anthraquinone administration. In the mouse intravenous injection study, samples were collected at 2, 10, 20, 40, 60, 120, 240, 360, and 600 minutes after anthraquinone administration. In the rat and mouse gavage studies, samples were collected at 30, 60, 120, 240, 480, 720, 1,080, and 1,440 minutes after anthraquinone administration. The samples were collected into tubes containing EDTA as an anticoagulant; the plasma was separated by centrifugation and stored at approximately –20° C until analysis.

All animals were observed twice daily for signs of morbidity and mortality. Individual body weights were recorded at randomization and on the day each animal was dosed (study day 1). Body weights from study day 1 were used for the calculation of dosing volumes.

Plasma samples were analyzed in the Bioanalytical Chemistry Laboratory using the techniques described in Appendix I for the determination of anthraquinone in plasma.

Individual replicate values were recorded and summarized as the mean ± standard deviation. The limit of quantitation (LOQ) was 0.025 μg/mL. If a measured concentration was less than 0.025 μg/mL, then a value of 0.0125 μg/mL (midpoint between 0 and 0.025 μg/mL) was used to calculate the mean.

Plasma concentration values are presented to two significant figures down to 0.01 μg/mL. Plasma concentration values were recorded for individual animals, and the mean ± standard deviation was calculated by gender, dose group, and time point using tables and graphic illustrations. Graphic illustrations include semilog plots of concentration versus time and area under the curve (AUC) versus dose. Values for AUC were calculated for each concentration-versus-time profile using the trapezoidal method. A software program (Sigma Plot, Version 5.0) was used to calculate the AUC values. Reported toxicokinetic parameters, i.e., C_{max}, T_{max}, and $t_{½}$, are observed values only.

RESULTS

Rats

The toxicokinetic parameters are observed values taken from the actual plasma concentration-time profiles. There was no attempt made to model the plasma concentration-time profile to obtain a best-fit curve. Semilogarithmic plasma concentration-versus-time graphs are shown in Figures N1 (intravenous administration) and N2 (oral administration). Observed toxicokinetic parameters are summarized in Table N5.

Intravenous Administration

The intravenous plasma concentration-time profiles appear to be biphasic curves for both male and female rats (Figure N1). A biphasic curve would suggest that anthraquinone is best described by a two-compartment open model. This model includes an initial tissue distribution phase (the initial portion of the biphasic curve) and an elimination phase (the terminal linear portion of the biphasic curve). The intravenous plasma concentration-time profiles have well defined distribution and elimination phases.

Observed toxicokinetic parameters obtained following the single intravenous bolus injection included a maximum anthraquinone plasma concentration (C_{max}) of approximately 2.9 ± 1.1 μg/mL (males) or 3.3 ± 1.8 μg/mL (females) at 2 minutes after dosing (T_{max}) (Table N5). The $t_{½}$, which was estimated by visual inspection of the semilogarithmic plasma concentration-time profile, was determined to be 10 to 12 hours for males and females. The AUC, calculated using the trapezoidal rule, was 1.29 μg/mL●min for males and 1.10 μg/mL●min for females.

Oral Gavage Administration

The plasma concentration-time profiles for anthraquinone following a single oral gavage administration were characteristic of a two-compartment open model with first order absorption and elimination (Figure N2). There was an initial upward phase that was used to characterize the absorption phase. Maximum plasma concentration and time to peak concentration were well defined for all male and female dose groups. The slow decreasing phase, or terminal linear portion, describes the elimination phase. The elimination phase was unclear in the 100 and 400 mg/kg male and female groups.

The observed C_{max} values were dose dependent and increased with increasing dose concentration (Table N5). The increase in C_{max} values was within acceptable limits to be considered proportional with dose. The observed T_{max} values were also dose dependent, increasing from 8 to 18 hours for males and females. The observed $t_{½}$ values were similar for the male and female 40 mg/kg groups. The $t_{½}$ values for the male and female 100 and 400 mg/kg groups could not be reliably estimated. Area under the plasma concentration-time profile (AUC) increased with increasing dose concentration for males and females. The increase was linear and appeared proportional (Figure N3).

Mice

Semilogarithmic plasma concentration-versus-time graphs are shown in Figures N4 (intravenous administration) and N5 (oral administration). Observed toxicokinetic parameters are summarized in Table N6.

Intravenous Administration

The intravenous plasma concentration-time profiles appear to be biphasic curves for both male and female mice (Figure N4). These profiles suggest that these data are best characterized by a two-compartment open model, with an initial tissue distribution phase and a terminal linear elimination phase.

Observed toxicokinetic parameters obtained following the single intravenous bolus injection (4 mg/kg) included a C_{max} of approximately 2.7 ± 1.2 μg/mL (males) or 3.4 ± 0.5 μg/mL (females) at 2 minutes after dosing (T_{max}) (Table N6). The $t_{½}$, which was estimated by visual inspection of the semilogarithmic plasma concentration-time profile, was determined to be 4 hours for males and females. The AUC, calculated by the trapezoidal rule from 0 to 10 hours, was 3.45 μg/mL•min for males and 2.16 μg/mL•min for females.

Oral Gavage Administration

Plasma concentration-time profiles for anthraquinone following a single oral gavage administration were characteristic of a two-compartment open model with first order absorption and elimination (Figure N5). There was an initial upward phase that was used to characterize the absorption phase. Maximum plasma concentration and time to peak concentration were well defined for all male and female dose groups. The later slow decreasing phase was observed and well defined in all dose groups except for the 800 mg/kg female group.

The observed C_{max} values were dose dependent and increased with increasing dose concentration (Table N6). The increase in C_{max} was proportional with dose for the male and female 80 and 200 mg/kg groups; however, the C_{max} for each 800 mg/kg group was lower than that expected based on the incremental increase in dose. The observed T_{max} values occurred at 4 hours for all groups. The observed $t_{½}$ was 4 to 6 hours for all dose groups. There was no evidence of saturation of elimination for anthraquinone between doses of 80 to 800 mg/kg for males and females. The area under the plasma concentration-time profile (AUC) increased with increasing dose, but the increase was not proportional with dose at the highest dose level.

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Voss, G.P. (1981). 9,10-Anthraquinone as an additive in chemical pulping. *Paper Technol. Ind.* **22**, 125-130.

TABLE N1
Plasma Concentrations of Anthraquinone in F344/N Rats after a Single Intravenous Injection of 2 mg/kg Anthraquinone[a]

	Concentration (μg/mL)
Male	
Time after dosing (minutes)	
2	2.88 ± 1.12
10	0.98 ± 0.12
20	0.48 ± 0.06
40	0.26 ± 0.04
60	0.18 ± 0.02
120	0.11 ± 0.03
300	0.08 ± 0.01
480	0.05 ± 0.01
600	0.03 ± 0.02[b]
Female	
Time after dosing (minutes)	
2	3.32 ± 1.76
10	0.64 ± 0.08
20	0.41 ± 0.05
40	0.20 ± 0.05
60	0.17 ± 0.03
120	0.08 ± 0.01
300	0.07 ± 0.01
480	0.04 ± 0.01
600	0.03 ± 0.02[b]

[a] Three animals were bled at each time point. Data are given as the mean ± standard deviation.
[b] Mean calculated using at least one value below the limit of quantitation (LOQ=0.025 μg/mL). For concentrations less than 0.025 μg/mL, a value of 0.0125 μg/mL (midpoint between 0 and 0.025 μg/mL) was used to calculate the mean.

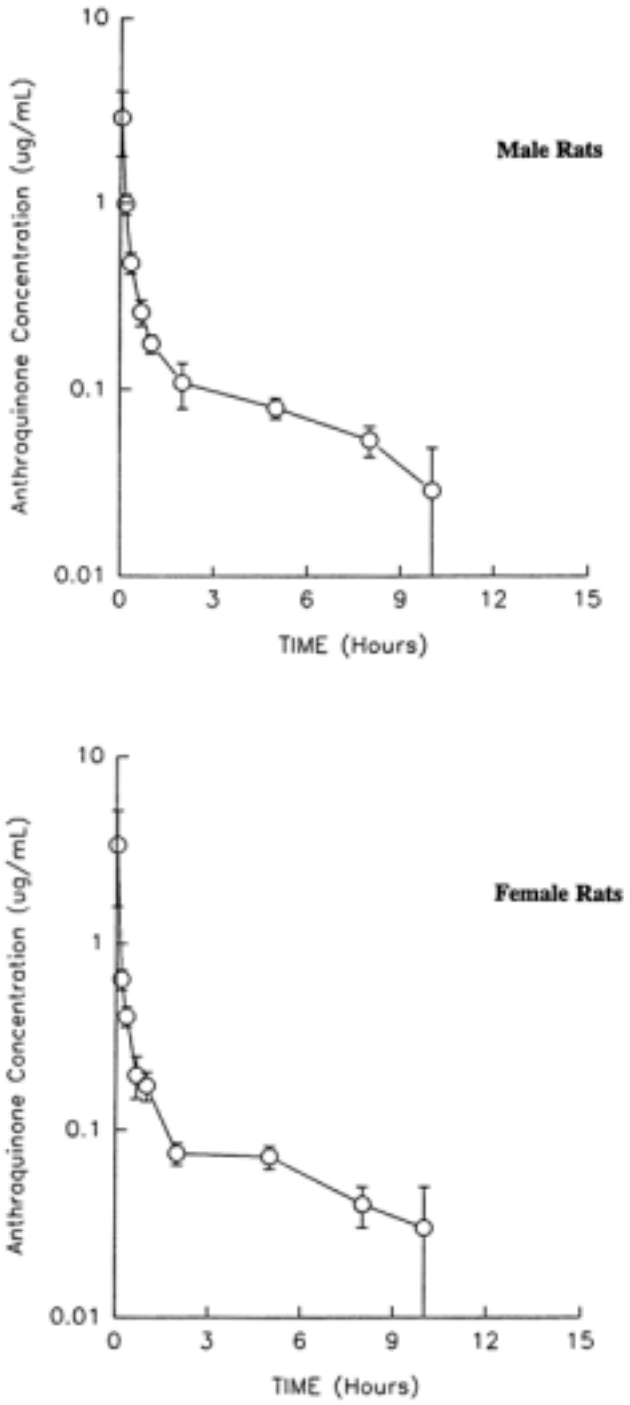

FIGURE N1
Plasma Concentrations of Anthraquinone in F344/N Rats after a Single Intravenous Injection of 2 mg/kg Anthraquinone

TABLE N2
Plasma Concentrations of Anthraquinone in F344/N Rats after a Single Gavage Dose of Anthraquinone[a]

	Dose		
	40 mg/kg	100 mg/kg	400 mg/kg
Male			
Time after dosing (minutes)			
30	0.01 ± 0.00[b]	0.01 ± 0.00[b]	0.14 ± 0.03
60	0.06 ± 0.00	0.06 ± 0.01	0.24 ± 0.11
120	0.12 ± 0.03	0.17 ± 0.06	0.29 ± 0.06
240	0.13 ± 0.02	0.26 ± 0.06	0.49 ± 0.12
480	0.26 ± 0.02	0.63 ± 0.12	1.71 ± 0.11
720	0.23 ± 0.01	0.70 ± 0.02	2.39 ± 0.28
1,080	0.10 ± 0.02	0.48 ± 0.10	2.63 ± 0.28
1,440	0.03 ± 0.03[b]	0.24 ± 0.03	2.01 ± 0.10
Female			
Time after dosing (minutes)			
30	0.01 ± 0.00[b]	0.03 ± 0.01	0.14 ± 0.04
60	0.04 ± 0.02	0.06 ± 0.00	0.20 ± 0.03
120	0.10 ± 0.01	0.18 ± 0.04	0.32 ± 0.09
240	0.12 ± 0.01	0.25 ± 0.02	0.48 ± 0.14
480	0.25 ± 0.05	0.77 ± 0.20	2.00 ± 0.43
720	0.23 ± 0.03	0.77 ± 0.14	2.89 ± 0.36
1,080	0.16 ± 0.03	0.55 ± 0.09	3.08 ± 0.75
1,440	0.03 ± 0.03[b]	0.33 ± 0.12	2.73 ± 0.30

[a] Three animals were bled at each time point. Data are given in μg/mL as the mean ± standard deviation.
[b] Mean calculated using at least one value below the limit of quantitation (LOQ=0.025 μg/mL). For concentrations less than 0.025 μg/mL, a value of 0.0125 μg/mL (midpoint between 0 and 0.025 μg/mL) was used to calculate the mean.

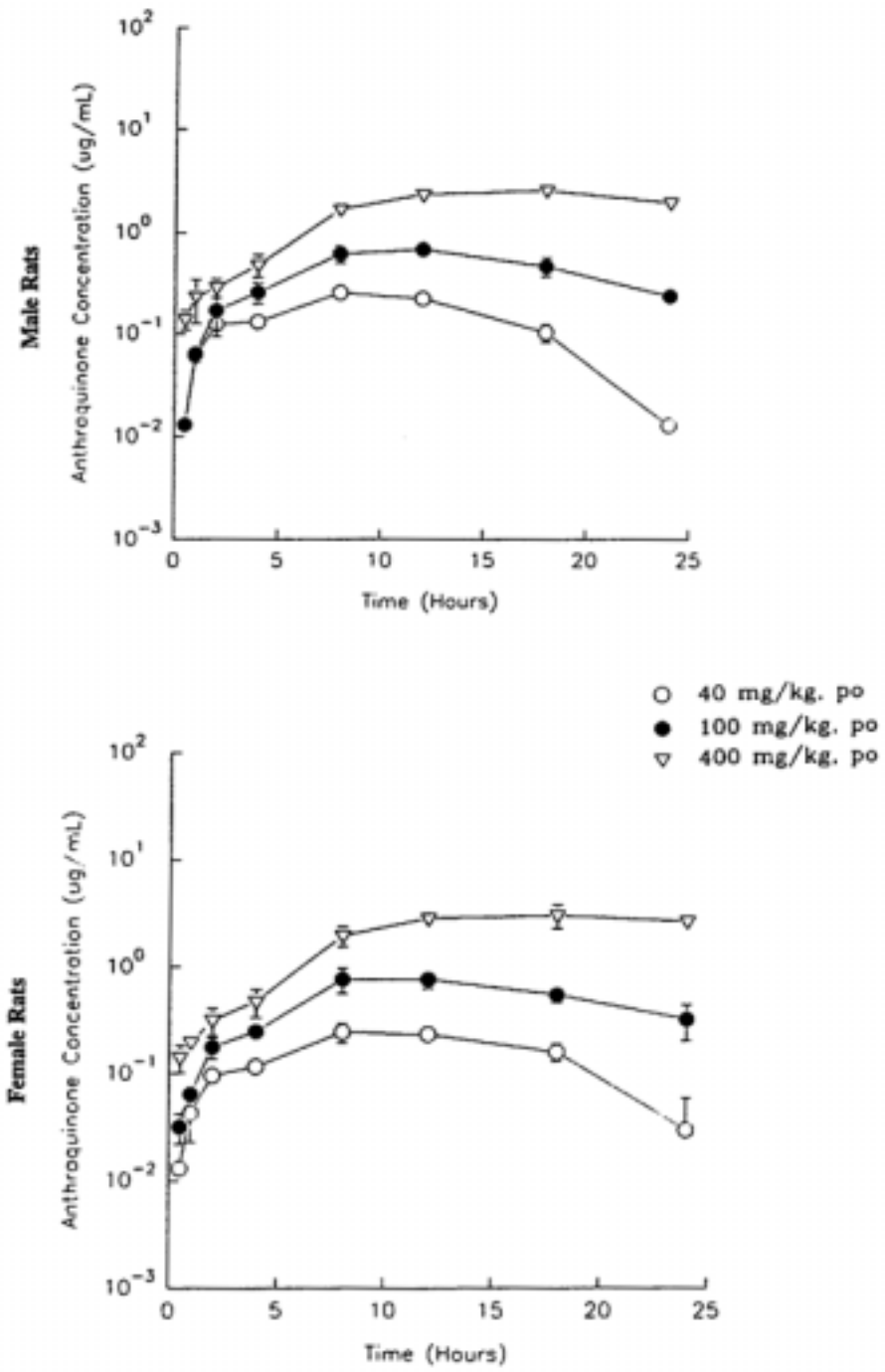

FIGURE N2
Plasma Concentrations of Anthraquinone in F344/N Rats after a Single Gavage Dose of Anthraquinone

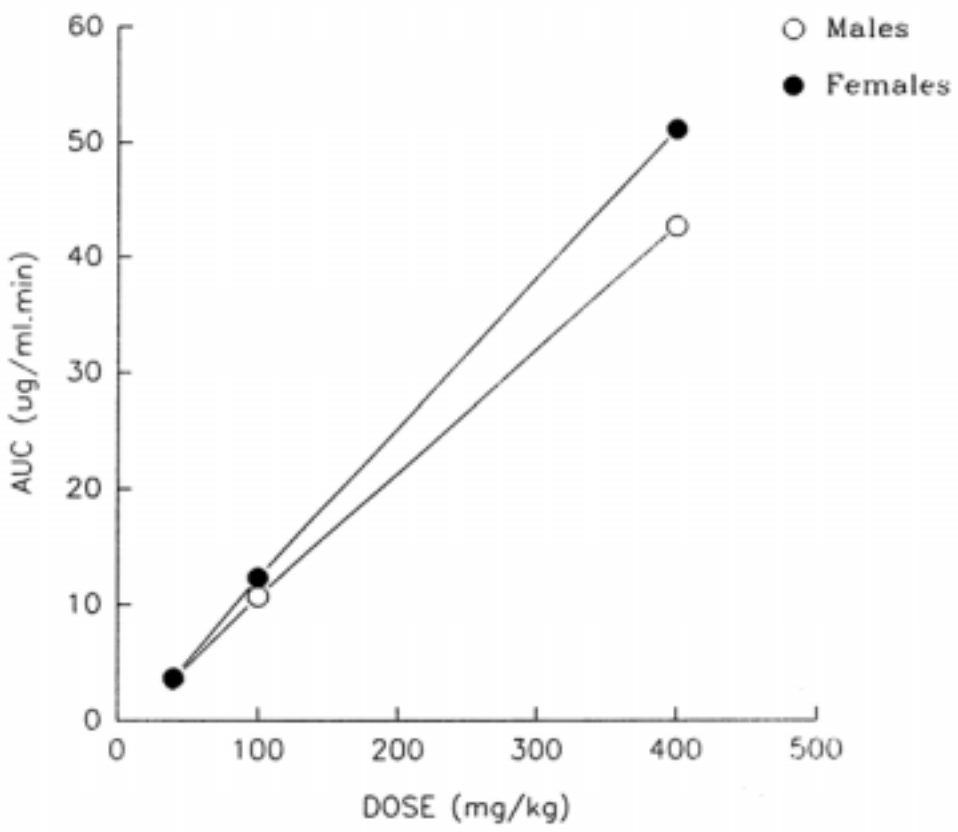

FIGURE N3
Dose Versus AUC for F344/N Rats after a Single Gavage Dose of Anthraquinone

TABLE N3
Plasma Concentrations of Anthraquinone in B6C3F$_1$ Mice after a Single Intravenous Injection of 4 mg/kg Anthraquinone[a]

	Concentration (μg/mL)
Male	
Time after dosing (minutes)	
2	2.73 ± 1.16
10	1.86 ± 0.06
20	1.38 ± 0.15
40	0.72 ± 0.33[b]
60	0.82 ± 0.12
120	0.33 ± 0.15
240	0.35 ± 0.09
360	0.21 ± 0.24[c]
600	0.01 ± 0.00[d]
Female	
Time after dosing (minutes)	
2	3.44 ± 0.54
10	1.59 ± 0.21
20	0.88 ± 0.11
40	0.55 ± 0.23
60	0.47 ± 0.14
120	0.29 ± 0.11
240	0.17 ± 0.02
360	0.06 ± 0.03
600	0.01 ± 0.00[d]

[a] Three animals were bled at each time point. Data are given as the mean ± standard deviation.
[b] Two animals bled
[c] Four animals bled
[d] Each replicate was below the limit of quantitation (LOQ=0.025 μg/mL). A value of 0.0125 μg/mL (midpoint between 0 and 0.025 μg/mL) was used for each replicate to calculate the mean.

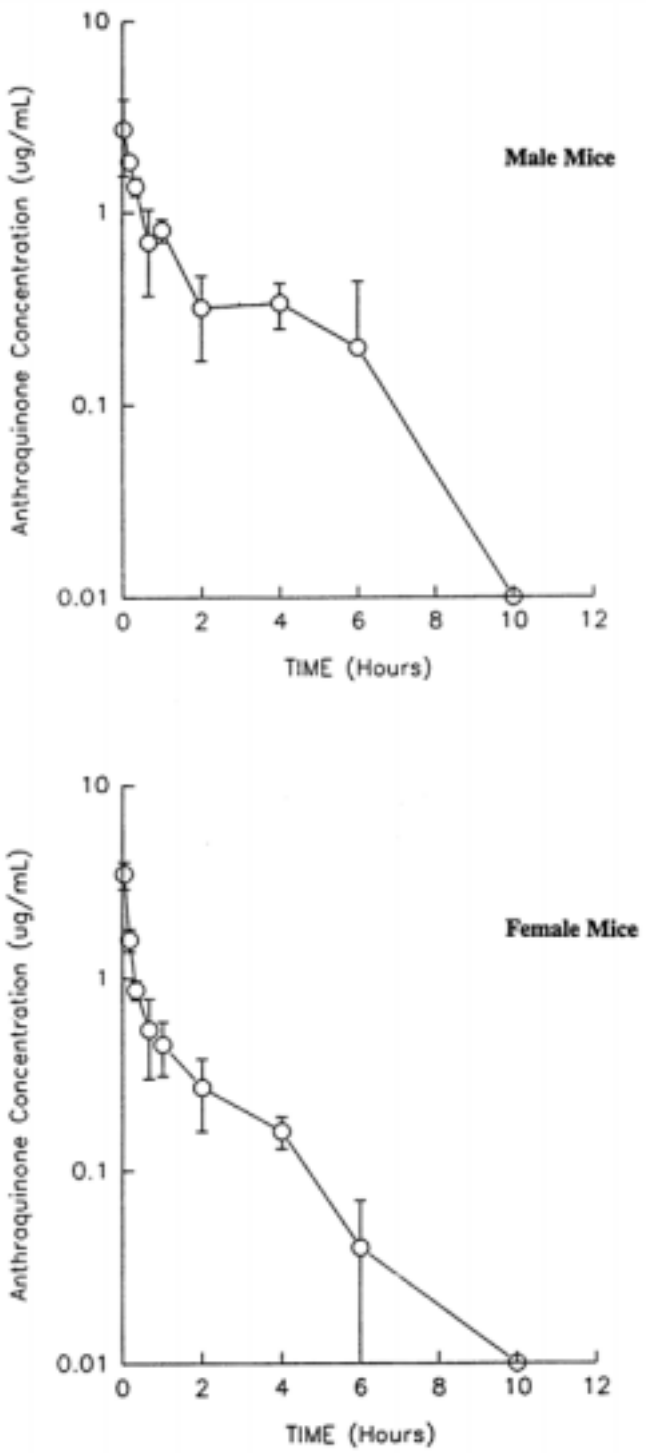

FIGURE N4
Plasma Concentrations of Anthraquinone in B6C3F$_1$ Mice after a Single Intravenous Injection of 4 mg/kg Anthraquinone

TABLE N4
Plasma Concentrations of Anthraquinone in B6C3F$_1$ Mice after a Single Gavage Dose of Anthraquinone[a]

	Dose		
	80 mg/kg	200 mg/kg	800 mg/kg
Male			
Time after dosing (minutes)			
30	0.08 ± 0.02	0.17 ± 0.02	0.51 ± 0.09
60	0.10 ± 0.04	0.17 ± 0.05	0.38 ± 0.04
120	0.53 ± 0.16	1.19 ± 0.11	2.36 ± 0.15
240	0.68 ± 0.17	2.11 ± 0.58	3.47 ± 1.07
480	0.14 ± 0.07	0.34 ± 0.17	1.57 ± 1.26
720	0.03 ± 0.02[b]	0.06 ± 0.02	0.25 ± 0.14
1,080	0.01 ± 0.00[b]	0.01 ± 0.00[b]	0.01 ± 0.00[b]
1,440	0.05 ± 0.06[b]	0.03 ± 0.01[b]	0.01 ± 0.00[b]
Female			
Time after dosing (minutes)			
30	0.13 ± 0.04	0.30 ± 0.10	0.69 ± 0.20
60	0.19 ± 0.07	0.34 ± 0.04	0.85 ± 0.37
120	0.48 ± 0.09	1.11 ± 0.16	2.43 ± 1.22[c]
240	0.71 ± 0.06	1.59 ± 0.15	2.63 ± 1.23
480	0.08 ± 0.03	0.28 ± 0.15	0.49 ± 0.13
720	0.01 ± 0.00[b]	0.01 ± 0.00[b]	0.27 ± 0.45[b]
1,080	0.01 ± 0.00[b]	0.05 ± 0.03[b]	0.01 ± 0.00[b]
1,440	0.01 ± 0.00[b]	0.01 ± 0.00[b]	0.01 ± 0.00[b]

[a] Three animals were bled at each time point. Data are given in μg/mL as the mean ± standard deviation.
[b] Mean calculated using at least one value below the limit of quantitation (LOQ=0.025 μg/mL). For concentrations less than 0.025 μg/mL, a value of 0.0125 μg/mL (midpoint between 0 and 0.025 μg/mL) was used to calculate the mean.
[c] Two animals bled

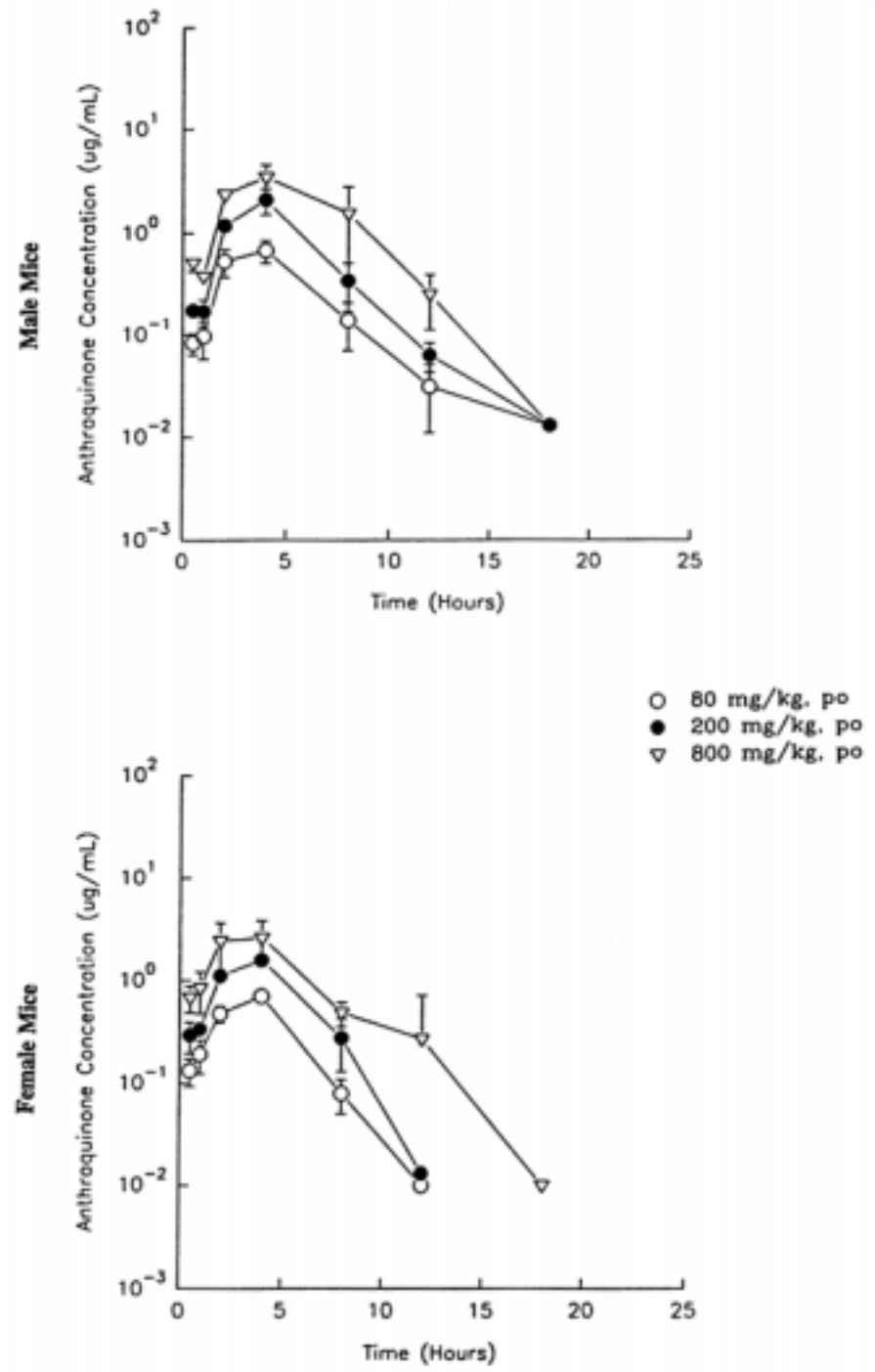

FIGURE N5
Plasma Concentrations of Anthraquinone in B6C3F$_1$ Mice after a Single Gavage Dose of Anthraquinone

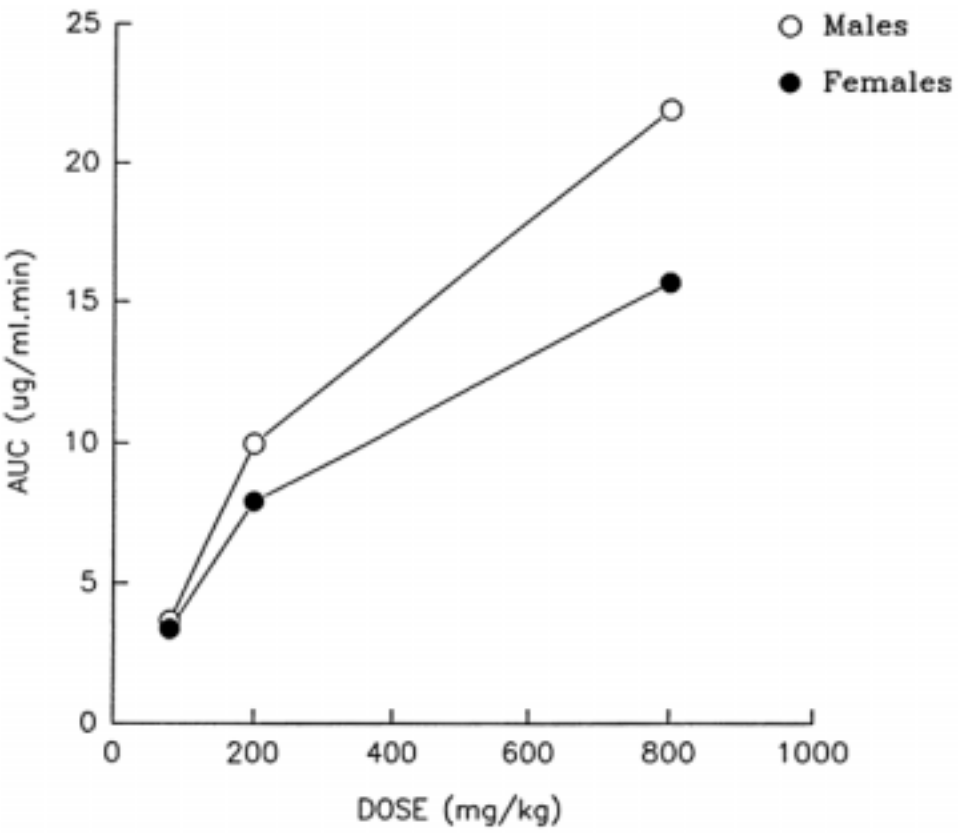

FIGURE N6
Dose Versus AUC for B6C3F$_1$ Mice after a Single Gavage Dose of Anthraquinone

TABLE N5
Summary of Toxicokinetic Data from a Single Dose Intravenous and Oral Gavage Anthraquinone Study in F344/N Rats[a]

Route	Dose (mg/kg)	C_{max}[b] (μg/mL)	T_{max} (hours)	$t_{½}$ (hours)	AUC (μg/mL•min)
Male					
Intravenous injection	2	2.88 ± 1.12	2[c]	10 to 12	1.29
Gavage	40	0.26 ± 0.02	8	12	3.54
Gavage	100	0.70 ± 0.02	12	ND[d]	10.7
Gavage	400	2.63 ± 0.28	18	ND	42.7
Female					
Intravenous injection	2	3.32 ± 1.76	2[c]	10 to 12	1.10
Gavage	40	0.25 ± 0.05	8	12	3.73
Gavage	100	0.77 ± 0.14	12	ND	12.3
Gavage	400	3.08 ± 0.75	18	ND	51.2

[a] C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration; $t_{½}$=elimination half-life; AUC=area under the curve calculated using the trapezoidal rule from 0 to 600 minutes.
[b] Data are given as the mean ± standard deviation.
[c] Minutes
[d] ND=not determined due to insufficient data

TABLE N6
Summary of Toxicokinetic Data from a Single Dose Intravenous and Oral Gavage Anthraquinone Study in B6C3F$_1$ Mice[a]

Route	Dose (mg/kg)	C_{max}[b] (μg/mL)	T_{max} (hours)	$t_{½}$ (hours)	AUC (μg/mL•min)
Male					
Intravenous injection	4	2.73 ± 1.16	2[c]	4	3.45
Gavage	80	0.68 ± 0.17	4	4 to 6	3.67
Gavage	200	2.11 ± 0.58	4	4 to 6	9.98
Gavage	800	3.47 ± 1.07	4	4 to 6	21.9
Female					
Intravenous injection	4	3.44 ± 0.54	2[c]	4	2.16
Gavage	80	0.71 ± 0.06	4	ND[d]	3.37
Gavage	200	1.59 ± 0.15	4	4 to 6	7.91
Gavage	800	2.63 ± 1.23	4	4 to 6	15.7

[a] C_{max}=maximum mean concentration; T_{max}=time of maximum mean concentration; $t_{½}$=elimination half-life; AUC=area under the curve calculated using the trapezoidal rule from 0 to 600 minutes.
[b] Data are given as the mean ± standard deviation.
[c] Minutes
[d] ND=not determined due to insufficient data

APPENDIX O
32-DAY FEED STUDY OF ANTHRAQUINONE IN F344/N RATS

INTRODUCTION O-2
MATERIALS AND METHODS O-2
RESULTS O-3
TABLE O1 Final Body Weights, Organ Weights, and Organ-Weight-to-Body-Weight Ratios for Rats in the 32-Day Feed Study of Anthraquinone O-5
TABLE O2 Liver Cytochrome P450 Activities for Rats at the 8-Day Interim Evaluation in the 32-Day Feed Study of Anthraquinone O-6
FIGURE O1 Liver Cytochrome P4501A1 Activities for Rats at the 8-Day Interim Evaluation in the 32-Day Feed Study of Anthraquinone O-7
FIGURE O2 Liver Cytochrome P4502B1 Activities at the 8-Day Interim Evaluation in the 32-Day Feed Study of Anthraquinone O-7
TABLE O3 Kidney and Liver 8-Hydroxy-2′-deoxyguanosine and 2′-Deoxyguanosine Concentrations for Rats at the 8-Day Interim Evaluation in the 32-Day Feed Study of Anthraquinone O-8
TABLE O4 Kidney, Liver, and Urinary Bladder Cell Proliferation Data for Rats in the 32-Day Feed Study of Anthraquinone O-9
FIGURE O3 Cell Proliferation in the Urinary Bladder of Rats in the 32-Day Feed Study of Anthraquinone O-9
TABLE O5 Incidences of Selected Nonneoplastic Lesions in Rats in the 32-Day Feed Study of Anthraquinone O-10

32-DAY FEED STUDY OF ANTHRAQUINONE IN F344/N RATS

INTRODUCTION

The 32-day feed study of anthraquinone in rats was designed to further evaluate the activity of anthraquinone at the sites where carcinogenic effects were observed in the 2-year study in rats: the liver, kidney, and urinary bladder. Cytochrome P450 activity in the liver, 8-hydroxy-2′-deoxyguanosine and 2′-deoxyguanosine concentrations in the liver and kidney, and cell proliferation in the liver, kidney, and urinary bladder were measured.

MATERIALS AND METHODS

Anthraquinone was obtained in one lot (5893) from Zeneca Fine Chemicals (Wilmington, DE), which was also used in the 14-week and 2-year studies. Identity, purity, and stability analyses of the bulk chemical are described in Appendix J; reanalyses by the gas chromatography system described for the initial purity analyses indicated that the bulk chemical remained stable throughout the 32-day study. The dosed feed mixtures were prepared and analyzed as described in Appendix J; all dose formulations and animal room samples were within 10% of the theoretical concentration. Solutions of 0.4 mg/mL bromodeoxyuridine (BrdU) in filtered water (Milli-Q® filtration system, Millipore Corp., Bedford, MA) were prepared by mixing a weighed amount of BrdU with water, diluting the mixture to the appropriate volume with additional water, and stirring for approximately 15 minutes or until the BrdU was dissolved.

F344/N rats, approximately 4 weeks of age, were obtained from Taconic Laboratory Animals and Services (Germantown, NY). The rats were quarantined for 12 days and were approximately 6 weeks old at the beginning of the study. Five male and five female rats were randomly selected for parasite evaluation and gross observation of disease. The health of the animals was monitored during the study according to the protocols of the NTP Sentinel Animal Program; results of all tests were negative. The rats received NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA) *ad libitum* except during urine collection periods; Milli-Q-filtered water was available *ad libitum*. The animals were housed five per cage in polycarbonate cages (Lab Products, Inc., Maywood, NJ) containing irradiated Sani-Chips® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ) for bedding; cages were rotated every 2 weeks, and cages and bedding were changed twice per week. The animal room was maintained at 69° to 75° F and 35% to 65% relative humidity, with at least 10 room air changes per hour and 12 hours of fluorescent light per day. The rats were observed twice per day. Animals were weighed weekly and at the end of the study. Feed consumption data were recorded weekly, and water consumption data were collected daily during the last 5 days of the study.

Groups of 20 male and 20 female rats were fed diets containing 0, 469, 938, or 3,750 ppm anthraquinone for up to 30 days over a 32-day period. Ten males and ten females from each group were designated for interim evaluation on day 8; the kidneys and livers of these rats were weighed, frozen in liquid nitrogen, and stored at or below −70° C for analyses of cytochrome P450 activities, 8-hydroxy-2′-deoxyguanosine concentrations, and 2′-deoxyguanosine concentrations. The remaining rats were fasted for approximately 16 hours for urine collection on days 16 and 17 of the study. Urine was collected over wet ice and protected from light. Urine samples for each group were pooled and centrifuged in a refrigerated centrifuge; the supernatants were flash frozen in liquid nitrogen prior to shipment to SRI International (Menlo Park, CA) for mutagenicity testing. During the last 5 days of the study, the rats received 0.4 mg/mL BrdU in drinking water. A necropsy was performed on all rats at the end of the study. The kidneys, liver, and urinary bladder were weighed; the duodenum was also removed for use as a positive control for cell proliferation. These organs, as well as all gross lesions, were fixed and preserved in 10% neutral buffered formalin and then transferred to 70% ethanol. Gross lesions and representative sections of each organ were processed and trimmed and embedded in paraffin. Two sections were prepared at a

thickness of 5 μm; one section was stained with hematoxylin and eosin prior to histopathologic examination, and the other section was stained with anti-BrdU antibody complexed with avidin and biotin for determinations of cell proliferation. BrdU-labeled and unlabeled cells in the kidney proximal tubules, liver, and urinary bladder mucosa were counted for approximately 2,000 nuclei per slide.

For the determination of liver cytochrome P450 activities, microsomes were prepared from the livers of rats evaluated on day 8. The microsomes were resuspended in storage buffer at a protein concentration of 7.7 to 19.6 mg/mL. On the day microsomes were prepared, total protein in the suspensions was measured by the Bradford Coomassie blue method with the addition of 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate. Two samples of each microsome suspension were stored at or below −60° C until analysis. The microsome suspensions were diluted with assay buffer to 5 mg protein/mL (control suspensions) or 1.7 mg/mL (exposed group suspensions) and analyzed with and without NADPH. The cytochrome P450-dependent formation of resorufin was measured by subtracting the relative fluorescence in the absence of NADPH from the relative fluorescence in the presence of NADPH; the activities of ethoxyresorufin-*O*-dealkylase and pentoxyresorufin-*O*-dealkylase determined from this measurement of resorufin formation were used to measure the induction of cytochrome P450 isoenzymes 1A1 and 2B1, respectively. Fluorescence was measured at 530 nm excitation (25 nm bandwidth) and 590 nm emission (35 nm bandwidth) with a sensitivity setting of 2. Standards of resorufin (lot 18H3639; Sigma Chemical Company, St. Louis, MO) in sodium phosphate buffer (pH 8) were used to prepare eight-point standard curves.

Concentrations of 8-hydroxy-2′-deoxyguanosine and 2′-deoxyguanosine were measured from the kidneys and livers of rats evaluated on day 8 by CEDRA Corporation (Austin, TX). DNA was isolated from kidney and liver samples with a commercially available kit (Genomic DNA Extraction/Nal Method, Wako Chemicals USA Inc., Richmond, VA). DNA was converted to nucleosides by treatment with nuclease and alkaline phosphate; 8-hydroxy-2′-deoxyguanosine was detected electrochemically with an ESA 5100A coulochem detector. An ultraviolet detector arranged in series with the coulochem detector was used to measure 2′-deoxyguanosine. A Zorbax SB-C_8 column (4.6 × 250 mm) was used; the flow rate was 1 mL/minute.

RESULTS

All animals not scheduled for the day 8 evaluation survived to the end of the study. The final mean body weights of all exposed groups of males were significantly less than the final mean body weight of the controls at the end of the study (Table O1). The final mean body weights of exposed and control females were similar. Dietary concentrations of 469, 938, and 3,750 ppm anthraquinone resulted in average daily doses of approximately 40, 80, and 320 mg/kg body weight to males and females.

At the 8-day interim evaluation, kidney and liver weights of all groups of exposed males and females were greater than those of the controls (Table O1). At the end of the study, kidney and liver weights of all groups of exposed females, relative liver and kidney weights of all groups of exposed males, and the absolute liver weight of males in the 3,750 ppm group were greater than those of the controls.

The activities of liver cytochrome P4501A1 were greater in exposed groups of males and females than in the controls at the 8-day interim evaluation, but the differences were not exposure concentration related (Table O2 and Figure O1). Activities of exposed animals were 2.7- to 3-fold (males) and 1.7- to 2.2-fold (females) greater than those of the controls. Cytochrome P4502B1 activities in males and females increased with increasing exposure concentration, with activities in the 3,750 ppm groups being 78-fold (males) and 48-fold (females) greater than those of the controls (Table O2 and Figure O2).

On day 8, concentrations of 8-hydroxy-2′-deoxyguanosine in the kidney of exposed males and females were slightly less than those in the controls, but the differences were not exposure concentration related (Table O3). Liver 8-hydroxy-2′-deoxyguanosine concentrations of exposed males and females were

variable. 2′-Deoxyguanosine concentrations in the kidney and liver were similar to those of the controls for males and less than those of the controls for females; 8-hydroxy-2′-deoxyguanosine/2′-deoxyguanosine ratios in the liver and kidney of males and females varied and did not show an exposure concentration response.

Cell proliferation values in the urinary bladder of males and females in the 3,750 ppm groups and females in the 938 ppm group were significantly greater than those of the controls (Table O4 and Figure O3). There were no significant differences in cell proliferation values in the liver or kidney of exposed males or females.

At the end of the study, all exposed males had hyaline droplets in the kidneys; females in the 938 and 3,750 ppm groups also had significantly increased incidences of hyaline droplets (Table O5). The severity of this lesion, which did not occur in control rats, was moderate in exposed males and minimal in exposed females. Males in the 938 and 3,750 ppm groups also had significantly higher incidences of minimal to mild nephropathy than the controls. In exposed males, hyaline droplets were glassy, stained brightly eosinophilic, and often filled the tubular epithelium or tubular lumens. The droplets were of varying size and often took on irregular shapes, including square and triangular forms. These droplets were consistent with α2u-globulin droplets, and their occurrence may have been related to hepatic enzyme activation resulting in the secretion of a protein by the male rat liver. Hyaline droplets in exposed females were much smaller than those in males and did not exhibit angular or irregular shapes, although they often filled the renal cell cytoplasm. Nephropathy in exposed males differed from that commonly seen in aging rats in that a greater number of affected nephrons were observed in a given section. Kidneys with one affected nephron in a given section were considered normal; kidneys with two to five affected nephrons were considered to exhibit minimal nephropathy; and kidneys with more than five affected nephrons were classified as having mild nephropathy.

All males and females in the 938 and 3,750 ppm groups had hypertrophy in the liver, and the incidence of this lesion was also significantly increased in males exposed to 469 ppm (Table O5). The severity of hypertrophy was mild in males exposed to 3,750 ppm and minimal in other groups of males and females. Three females in the 469 ppm group also had mild hypertrophy. Hypertrophy consisted of minimal to mild enlargement of the hepatocytes due to an increase in the amount of cytoplasm. The cytoplasm was eosinophilic with a grainy or ground-glass appearance. Some separation of basophilic cellular organelles within the cytoplasm occurred in affected cells. In rats with minimal hypertrophy, centrilobular cells were slightly larger than those of control males, and hypertrophied cells often extended halfway to the portal triad. Mild hypertrophy extended up to the portal triad in most areas of the liver.

TABLE O1
Final Body Weights, Organ Weights, and Organ-Weight-to-Body-Weight Ratios for Rats in the 32-Day Feed Study of Anthraquinone[a]

	0 ppm	469 ppm	938 ppm	3,750 ppm
n	10	10	10	10
Male				
8-Day Interim Evaluation				
Final body wt	164 ± 4	167 ± 4	159 ± 4	168 ± 4
L. and R. Kidney				
Absolute	1.572 ± 0.040	1.742 ± 0.054*	1.648 ± 0.046	1.778 ± 0.040**
Relative	9.56 ± 0.09	10.41 ± 0.11**	10.35 ± 0.13**	10.56 ± 0.17**
Liver				
Absolute	8.38 ± 0.31	9.77 ± 0.31*	10.06 ± 0.36**	11.72 ± 0.31**
Relative	50.85 ± 0.98	58.43 ± 1.14**	63.09 ± 0.99**	69.66 ± 1.48**
32-Day Study				
Final body wt	266 ± 5	237 ± 12**	230 ± 3**	241 ± 5*
L. and R. Kidney				
Absolute	2.270 ± 0.051	2.170 ± 0.105	2.080 ± 0.035*	2.232 ± 0.058
Relative	8.54 ± 0.11	9.17 ± 0.10**	9.05 ± 0.08**	9.26 ± 0.13**
Liver				
Absolute	14.27 ± 0.46	13.94 ± 0.90	15.01 ± 0.24	16.63 ± 0.39**
Relative	53.63 ± 1.24	58.24 ± 1.77*	65.33 ± 0.73**	69.05 ± 0.89**
Urinary Bladder				
Absolute	0.073 ± 0.003	0.068 ± 0.004	0.063 ± 0.003	0.082 ± 0.004
Relative	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.34 ± 0.02**
Female				
8-Day Interim Evaluation				
Final body wt	125 ± 2	123 ± 2	124 ± 2	123 ± 2
L. and R. Kidney				
Absolute	1.187 ± 0.037	1.267 ± 0.029	1.262 ± 0.034	1.240 ± 0.023
Relative	9.48 ± 0.15	10.32 ± 0.12**	10.17 ± 0.13**	10.11 ± 0.14**
Liver				
Absolute	5.645 ± 0.202	6.363 ± 0.152**	6.988 ± 0.159**	7.941 ± 0.090**
Relative	45.08 ± 1.03	51.88 ± 0.97**	56.42 ± 1.00**	64.79 ± 0.80**
32-Day Study				
Final body wt	165 ± 3	164 ± 3	164 ± 3	158 ± 2
L. and R. Kidney				
Absolute	1.405 ± 0.026	1.564 ± 0.040**	1.588 ± 0.029**	1.549 ± 0.034**
Relative	8.52 ± 0.10	9.52 ± 0.13**	9.72 ± 0.12**	9.79 ± 0.18**
Liver				
Absolute	6.991 ± 0.134	8.357 ± 0.230**	9.906 ± 0.240**	9.890 ± 0.162**
Relative	42.43 ± 0.66	50.84 ± 0.78**	60.58 ± 1.05**	62.57 ± 1.00**
Urinary Bladder				
Absolute	0.053 ± 0.002	0.058 ± 0.002	0.060 ± 0.003	0.060 ± 0.002
Relative	0.32 ± 0.01	0.36 ± 0.02	0.36 ± 0.01	0.38 ± 0.02*

* 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 O2
Liver Cytochrome P450 Activities for Rats at the 8-Day Interim Evaluation in the 32-Day Feed Study of Anthraquinone[a]

	0 ppm	469 ppm	938 ppm	3,750 ppm
n	10	10	10	10
Male				
Cytochrome P4501A1	19.4 ± 1.6	58.0 ± 4.0	56.3 ± 5.8	52.1 ± 4.4
Cytochrome P4502B1	3.3 ± 0.3	131 ± 13	215 ± 10[b]	257 ± 8
Female				
Cytochrome P4501A1	25.3 ± 2.4	55.7 ± 5.8	42.5 ± 6.1	46.8 ± 4.6
Cytochrome P4502B1	3.0 ± 0.4	41.4 ± 4.8	96.2 ± 9.6	143 ± 15

[a] Data are given as pmol/minute per mg protein (mean ± standard error).
[b] n=9

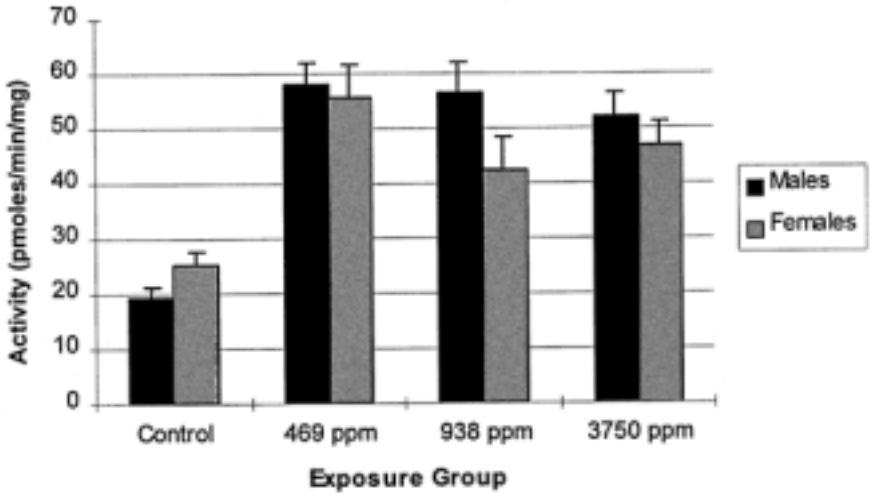

FIGURE O1
Liver Cytochrome P4501A1 Activities for Rats at the 8-Day Interim Evaluation in the 32-Day Feed Study of Anthraquinone

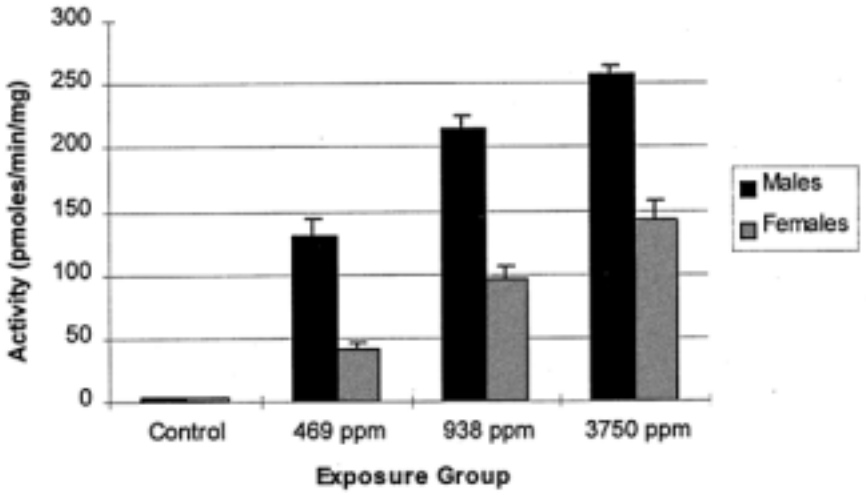

FIGURE O2
Liver Cytochrome P4502B1 Activities for Rats at the 8-Day Interim Evaluation in the 32-Day Feed Study of Anthraquinone

TABLE O3
Kidney and Liver 8-Hydroxy-2′-deoxyguanosine and 2′-Deoxyguanosine Concentrations for Rats at the 8-Day Interim Evaluation in the 32-Day Feed Study of Anthraquinone[a]

	0 ppm	469 ppm	938 ppm	3,750 ppm
Male				
n	10	10	10	7
Kidney				
8-Hydroxy-2′-deoxyguanosine (pg)	22.6 ± 8.5[b]	20.5 ± 3.7[c]	16.8 ± 1.7[d]	20.0 ± 2.4
2′-Deoxyguanosine (ng)	1,260 ± 140	1,360 ± 60	1,200 ± 80	1,210 ± 120[e]
8-Hydroxy-2′-deoxyguanosine/ 2′-deoxyguanosine ratio	1.70 ± 0.76[b]	1.41 ± 0.22[c]	1.36 ± 0.15[d]	1.54 ± 0.28
Liver				
8-Hydroxy-2′-deoxyguanosine (pg)	21.5 ± 2.5	25.2 ± 3.4[b]	18.1 ± 2.2[b]	33.4 ± 11.9
2′-Deoxyguanosine (ng)	1,440 ± 120	1,440 ± 150	1,450 ± 120	1,420 ± 180[e]
8-Hydroxy-2′-deoxyguanosine/ 2′-deoxyguanosine ratio	1.45 ± 0.16	1.62 ± 0.20[b]	1.14 ± 0.13[b]	2.14 ± 0.77
Female				
n	10	10	10	10
Kidney				
8-Hydroxy-2′-deoxyguanosine (pg)	25.6 ± 2.6[f]	24.5 ± 4.0[d]	20.6 ± 2.7[f]	23.3 ± 2.2[c]
2′-Deoxyguanosine (ng)	2,020 ± 130	1,890 ± 130	1,930 ± 90	1,540 ± 200
8-Hydroxy-2′-deoxyguanosine/ 2′-deoxyguanosine ratio	1.20 ± 0.11[f]	1.26 ± 0.20[d]	0.98 ± 0.12[f]	1.30 ± 0.11[c]
Liver				
8-Hydroxy-2′-deoxyguanosine (pg)	30.2 ± 4.2	41.0 ± 8.2	28.1 ± 4.6	25.5 ± 4.5[f]
2′-Deoxyguanosine (ng)	2,200 ± 130	2,140 ± 200	2,180 ± 80	1,740 ± 150
8-Hydroxy-2′-deoxyguanosine/ 2′-deoxyguanosine ratio	1.33 ± 0.21	1.77 ± 0.27	1.18 ± 0.16	1.37 ± 0.18[f]

[a] Data are given as mean ± standard error. Concentrations of 8-hydroxy-2′-deoxyguanosine and 2′-deoxyguanosine are values on column.
[b] n=7
[c] n=6
[d] n=8
[e] n=10
[f] n=9

TABLE O4
Kidney, Liver, and Urinary Bladder Cell Proliferation Data for Rats in the 32-Day Feed Study of Anthraquinone[a]

	0 ppm	469 ppm	938 ppm	3,750 ppm
n	10	10	10	10
Male				
Kidney	9.39 ± 0.72	7.73 ± 0.87	8.58 ± 0.56	10.4 ± 1.2
Liver	5.81 ± 0.88	5.68 ± 1.06	6.38 ± 0.95	7.56 ± 1.05
Urinary bladder	0.495 ± 0.077	0.840 ± 0.233[b]	0.532 ± 0.087	1.50 ± 0.24*
Female				
Kidney	4.75 ± 0.27	5.22 ± 0.40	4.82 ± 0.45	4.47 ± 0.35
Liver	3.45 ± 0.47	5.57 ± 1.22	6.54 ± 0.87	3.85 ± 0.66
Urinary bladder	1.07 ± 0.13[b]	2.60 ± 0.43[b]	4.96 ± 0.81*	3.15 ± 0.44*

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's one-tailed *t*-test
[a] Data are given as the percentage of mean labeled cells (mean ± standard error).
[b] n=9

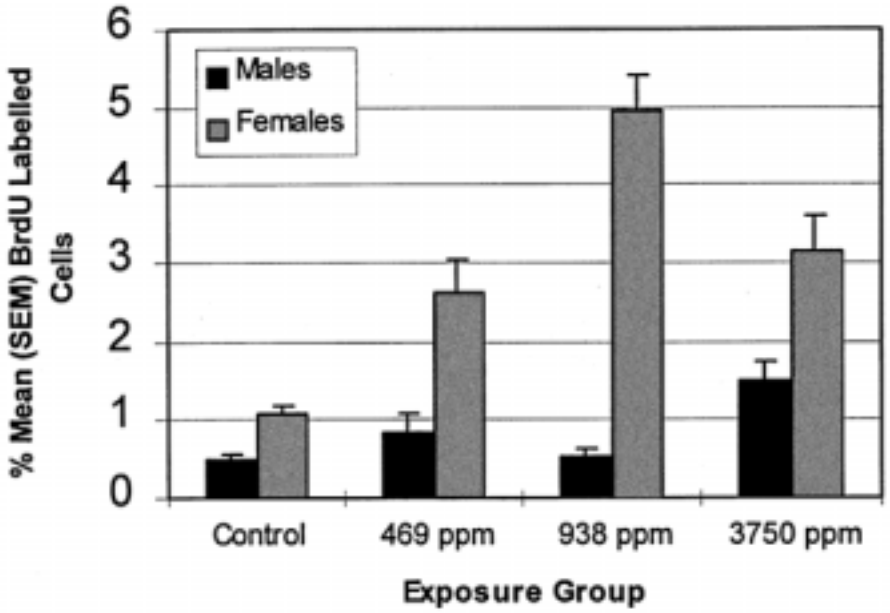

FIGURE O3
Cell Proliferation in the Urinary Bladder of Rats in the 32-Day Feed Study of Anthraquinone

TABLE O5
Incidences of Selected Nonneoplastic Lesions in Rats in the 32-Day Feed Study of Anthraquinone

	0 ppm	469 ppm	938 ppm	3,750 ppm
Male				
Liver[a]	10	10	10	10
Hypertrophy[b]	0	9** (1.1)[c]	10** (1.2)	10** (1.7)
Kidney	10	10	10	10
Hyaline Droplets	0	10** (2.5)	10** (3.0)	10** (3.0)
Nephropathy	3 (1.0)	7 (1.3)	9** (1.3)	10** (1.7)
Female				
Liver	10	10	10	10
Hypertrophy	0	3 (1.7)	10** (1.2)	10** (1.4)
Kidney	10	10	10	10
Hyaline Droplets	0	2 (1.0)	6** (1.0)	9** (1.0)
Nephropathy	3 (1.0)	1 (1.0)	1 (1.0)	1 (1.0)

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