NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 465

TOXICOLOGY AND CARCINOGENESIS STUDIES OF PHENOLPHTHALEIN

(CAS NO. 77-09-8)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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. 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.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

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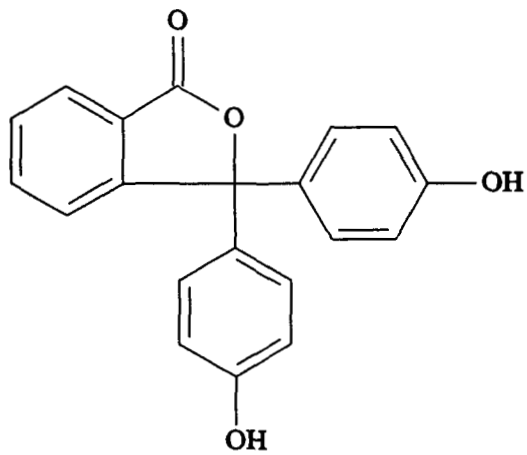
ABSTRACT



PHENOLPHTHALEIN

CAS No. 77-09-8

Chemical Formula: $C_{20}H_{14}O_4$ Molecular Weight: 318.33

Synonyms: 3,3-Bis(4-hydroxyphenyl)-1(3H)-isobenzofuranone; 3,3-bis(*p*-hydroxyphenyl)phthalide; α-(*p*-hydroxyphenyl)-α-(4-oxo-2,5-cyclohexadien-1-ylidene)-*o*-toluic acid

Trade names: Agoral®, Alophen®, Colax®, Correctol®, Dialose®, Doxidan®, Espotabs®, Evac-U-Gen®, Evac-U-Lax®, Ex-Lax®, Feen-A-Mint®, FemiLax®, Kondremul®, LaxCaps®, Lax-Pills®, Medilax®, Modane®, Phenolax®, Prulet®

Phenolphthalein is used as a laboratory reagent and acid-base indicator and in over-the-counter laxative preparations. The National Cancer Institute nominated phenolphthalein for study because of its widespread use as a component in numerous laxative preparations and the lack of adequate testing for carcinogenicity in experimental animals. Male and female F344/N rats and B6C3F$_1$ mice were exposed to phenolphthalein (98% to 99% pure) in feed for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and mouse peripheral blood.

14-DAY STUDY IN RATS

Groups of five male and five female F344/N rats were given 0, 6,250, 12,500, 25,000, 50,000, or 100,000 ppm phenolphthalein in feed for 14 days. All rats survived to the end of the study. The final mean body weights of all exposed groups of rats were similar to those of the controls. No chemical-related gross or microscopic lesions were observed.

14-DAY STUDY IN MICE

Groups of five male and five female B6C3F$_1$ mice were given 0, 6,250, 12,500, 25,000, 50,000, or

100,000 ppm phenolphthalein in feed for 14 days. All mice survived to the end of the study. The final mean body weights of all exposed groups of mice were similar to those of the controls. No chemical-related gross or microscopic lesions were observed.

13-Week Study in Rats

Groups of 10 male and 9 or 10 female F344/N rats were given 0, 3,000, 6,000, 12,000, 25,000, or 50,000 ppm phenolphthalein (equivalent to average daily doses of approximately 200, 400, 800, 1,600, or 3,500 mg phenolphthalein/kg body weight to males and 200, 400, 800, 1,700, or 3,600 mg/kg to females) in feed for 13 weeks. Additional groups of 10 male and 10 female rats designated for clinical pathology evaluations were also given 0, 3,000, 6,000, 12,000, 25,000, or 50,000 ppm phenolphthalein in feed until day 21. All core study rats survived to the end of the study. The final mean body weight of the 50,000 ppm females and the mean body weight gains of the 25,000 and 50,000 ppm females were significantly lower than those of the controls. The final mean body weights and mean body weight gains of all other exposed groups were similar to those of the controls. There was no cathartic action or any other clinical finding attributed to exposure to phenolphthalein. The few differences in the hematology and clinical chemistry parameters were sporadic and were not considered to be chemical related. The percentage of motile sperm in the 12,000 ppm males was significantly greater than that in the controls, but no other significant differences in sperm morphology or vaginal cytology between exposed and control groups were observed. Absolute and relative liver weights of 25,000 and 50,000 ppm males were significantly greater than those of the controls. No chemical-related gross or microscopic lesions were observed.

13-Week Study in Mice

Groups of 10 male and 10 female B6C3F$_1$ mice were given 0, 3,000, 6,000, 12,000, 25,000, or 50,000 ppm phenolphthalein (equivalent to average daily doses of approximately 500, 1,000, 2,000, 4,100, or 9,000 mg phenolphthalein/kg body weight to males and 600, 1,200, 2,400, 5,000, or 10,500 mg/kg to females) in feed for 13 weeks. All mice survived until the end of the study. The final mean body weights and mean body weight gains of all exposed groups were similar to those of the controls. There was no cathartic action or any other clinical finding attributed to exposure to phenolphthalein. The absolute right cauda weight of the 12,000 ppm males and the absolute right epididymis weights of 12,000, 25,000, and 50,000 ppm males were significantly less than those of the controls. The percentages of abnormal sperm in 12,000, 25,000, and 50,000 ppm males were significantly greater than that in the control group, and the sperm concentrations in 12,000 and 50,000 ppm males were significantly less than that of the control group. The absolute and relative right testis weights of males exposed to 6,000 ppm or greater and the absolute right testis weight of 3,000 ppm males were significantly less than those of the controls. The incidences of hypoplasia of the bone marrow in males and females exposed to 12,000 ppm or greater were significantly greater than those in the controls. The incidences of hematopoiesis of the spleen in 25,000 and 50,000 ppm males were significantly greater than that in the controls.

2-Year Study in Rats

Groups of 50 male and 50 female F344/N rats were given 0, 12,000, 25,000, or 50,000 ppm phenolphthalein (equivalent to average daily doses of approximately 500, 1,000, or 2,000 mg phenolphthalein/kg body weight to males and 500, 1,000, or 2,500 mg/kg to females) in feed for 2 years.

Survival, Body Weights, and Clinical Findings

Survival of exposed males and females was similar to that of the controls. The mean body weights of exposed males were less than those of the controls through most of the second year of the study, and the mean body weights of exposed females were less than those of the controls from about week 16 until the end of the study. Clinical findings attributed to phenolphthalein exposure included thin appearance and ruffled fur in all exposed groups of males.

Determinations of Total Phenolphthalein in Plasma

The mean plasma concentrations of total phenolphthalein (free and conjugated) after 2 years of exposure varied little with time of day. Plasma concentrations of total phenolphthalein were approximately the same between exposure groups and between males and females.

Pathology Findings

The incidences of benign pheochromocytoma of the adrenal medulla in all exposed groups of males were significantly greater than those in the controls and occurred with a significant positive trend. The incidences of benign pheochromocytoma in 12,000 ppm females and of benign or malignant pheochromocytoma (combined) in 12,000 and 25,000 ppm females were significantly greater than those in the controls. The numbers of exposed males with bilateral benign pheochromocytomas exceeded the number of controls with these neoplasms. The incidences of malignant pheochromocytomas in exposed rats were similar to those in the controls. The incidences of focal hyperplasia of the adrenal medulla in the 12,000 and 50,000 ppm males were significantly greater than in the controls.

The incidences of renal tubule adenoma in 50,000 ppm male rats and of renal tubule adenoma or carcinoma (combined) in 12,000 and 50,000 ppm male rats were significantly greater than those in the controls. Although the increased incidences were predominantly of renal tubule adenoma, four carcinomas were observed in exposed males (0 ppm, 0/50; 12,000 ppm, 1/50; 25,000 ppm, 1/50; 50,000 ppm, 2/50). The incidences of renal tubule neoplasms in exposed groups of females were similar to those in the controls. The findings from an extended evaluation (step section) of the kidneys of female rats were similar to those from the standard evaluation. The incidences of nephropathy in all exposed groups of females were significantly greater than in the controls, and the severity of nephropathy in all exposed groups of males and in 25,000 and 50,000 ppm females was significantly greater than in the controls.

The incidences of diffuse hyperplasia of the parathyroid gland (0/41, 16/48, 14/49, 14/46), fibrous osteodystrophy of the bone (0/50, 17/50, 14/50, 12/50), and mineralization (0/50, 11/50, 5/50, 5/49) and degeneration (0/50, 11/50, 5/50, 4/49) of the glandular stomach in exposed groups of males were generally significantly greater than those in the controls. The incidences of hyperplasia of the thyroid gland C-cells (13/50, 3/50, 9/49, 4/49) in 12,000 and 50,000 ppm males were significantly less than in the controls. These lesions are commonly observed in male rats with more advanced nephropathy and are considered to be associated with a calcium/phosphorus imbalance created by compromised functional capacity of the kidney.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female B6C3F$_1$ mice were given 0, 3,000, 6,000, or 12,000 ppm phenolphthalein (equivalent to average daily doses of approximately 300, 600, or 1,200 mg phenolphthalein/kg body weight to males and 400, 800, or 1,500 mg/kg to females) in feed for 2 years.

Survival, Body Weights, and Clinical Findings

Survival of the 12,000 ppm females was significantly lower than that of the controls; survival of all other exposed groups of mice was similar to that of the controls. The mean body weights of 12,000 ppm males were slightly less than those of the controls beginning at week 93 of the study, and the mean body weights of the 3,000, 6,000, and 12,000 ppm females were less than those of the controls during most of the second year of the study. In exposed mice, there were no clinical findings related to phenolphthalein exposure.

Determinations of Total Phenolphthalein in Plasma

The mean plasma concentrations of total phenolphthalein (free and conjugated) after 2 years of exposure varied little with time of day. Plasma concentrations of total phenolphthalein were approximately the same between exposure groups and between males and females.

Pathology Findings

The incidences of histiocytic sarcoma in 6,000 and 12,000 ppm males and females were significantly greater than those in the controls and occurred with a significant positive trend. In this study, histiocytic sarcoma was consistently observed in the liver with several other sites (e.g., spleen, lung, bone marrow, and various lymph nodes) involved less frequently.

The incidences of all types of malignant lymphoma and of lymphoma of thymic origin in all exposed groups of females were significantly greater than those in the controls and occurred with significant positive trends, while the incidences of all types of malignant lymphoma in all exposed groups of males were similar to that in the controls. The incidences of lymphoma of thymic origin were increased in exposed groups of males, but were significantly increased only in the 6,000 ppm group. The incidences of atypical hyperplasia of the thymus in 6,000 and 12,000 ppm males and in all exposed groups of females were significantly greater than those in the controls.

The incidences of benign sex-cord stromal tumors of the ovary in all exposed groups of females were significantly greater than in the controls. The incidences of hyperplasia of the ovary in 3,000 and 12,000 ppm females were significantly greater than in the controls. The incidences of germinal epithelial degeneration of the testis in all exposed groups of males were significantly greater than that in the controls.

There were increased incidences of myelofibrosis of the bone marrow in 12,000 ppm males (0 ppm, 3/50; 3,000 ppm, 8/50; 6,000 ppm, 8/50; 12,000 ppm, 19/49) and an increased severity but not incidence of this lesion in exposed females. There were also increased incidences of pigmentation of minimal to mild severity in the bone marrow of 6,000 and 12,000 ppm males (0/50, 2/50, 5/50, 16/49) and females (2/50, 3/50, 11/50, 11/50).

Also, the incidences of hematopoietic cell proliferation in the red pulp of the spleen (10/50, 22/50, 28/50, 21/49) in all exposed groups of males were significantly greater than that in the controls, and the severity of this lesion increased with increasing exposure concentration.

The incidences of hepatocellular adenoma in all exposed groups of males and females and of hepatocellular adenoma or carcinoma (combined) in 6,000 and 12,000 ppm males and all exposed groups of females were significantly less than those in the controls, and these lesions occurred with significant negative trends. Multiple hepatocellular adenomas were observed more frequently in the control groups than in the exposed groups. The incidences of clear cell and eosinophilic foci in all exposed groups of males and of mixed cell foci in 12,000 ppm males were significantly less than those in the controls. The incidences of eosinophilic foci in exposed groups of females were significantly less than in the controls.

GENETIC TOXICOLOGY

Phenolphthalein, tested in two laboratories, was not mutagenic in any of four strains of *Salmonella typhimurium* with or without S9 metabolic activation enzymes, and no induction of sister chromatid exchanges was observed in cultured Chinese hamster ovary cells treated with phenolphthalein with or without S9. However, significant increases in chromosomal aberrations were observed after treatment of cultured Chinese hamster ovary cells with phenolphthalein in the presence of S9, and the frequencies of micronucleated erythrocytes were increased in peripheral blood samples from male and female mice administered phenolphthalein in feed for 13 weeks.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity** of phenolphthalein in male F344/N rats based on markedly increased incidences of benign pheochromocytomas of the adrenal medulla and of renal tubule adenomas and adenomas or carcinomas (combined). There was *some evidence of carcinogenic activity* of phenolphthalein in female F344/N rats based on the increased incidences of benign pheochromocytomas of the adrenal medulla in the 12,000 ppm group and of benign or malignant pheochromocytomas (combined) in the 12,000 and 25,000 ppm groups. There was *clear evidence of carcinogenic activity* of phenolphthalein in male B6C3F$_1$ mice based on increased incidences of histiocytic sarcomas and of malignant lymphomas of thymic origin. There was *clear evidence of carcinogenic activity* of phenolphthalein in female B6C3F$_1$ mice based on increased incidences of histiocytic sarcomas, malignant lymphomas of all types, lymphomas of thymic origin, and benign sex-cord stromal tumors of the ovary.

Exposure of rats to phenolphthalein in feed for 2 years resulted in increased incidences of focal hyperplasia of the adrenal medulla in males and in increased incidences and/or severity of nephropathy of the kidney in males and females. Exposure of mice to phenolphthalein in feed for 2 years resulted in increased incidences of atypical hyperplasia of the thymus in males and females, degeneration of the germinal epithelium of the testis in males, and ovarian hyperplasia in females.

Exposure of mice to phenolphthalein in feed for 2 years resulted in decreased incidences of hepatocellular neoplasms and nonneoplastic lesions in males and females.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

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

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 12,000, 25,000, or 50,000 ppm in feed (approximately 500, 1,000, or 2,000 mg/kg per day)	0, 12,000, 25,000, or 50,000 ppm in feed (approximately 500, 1,000, or 2,500 mg/kg per day)	0, 3,000, 6,000, or 12,000 ppm in feed (approximately 300, 600, or 1,200 mg/kg per day)	0, 3,000, 6,000, or 12,000 ppm in feed (approximately 400, 800, or 1,500 mg/kg per day)
Body weights	Exposed groups lower than the control group	Exposed groups lower than the control group	12,000 ppm group slightly lower than the control group	Exposed groups lower than the control group
2-Year survival rates	21/50, 15/50, 15/50, 13/50	30/50, 38/50, 32/50, 38/50	40/50, 33/50, 36/50, 36/49	39/50, 31/50, 34/50, 28/50
Nonneoplastic effects	Adrenal medulla: focal hyperplasia (13/50, 22/50, 18/50, 23/50) Kidney: severity of nephropathy (1.8, 2.9, 3.1, 3.1)	Kidney: nephropathy (34/50, 45/50, 43/50, 44/50); severity of nephropathy (1.2, 1.4, 1.5, 1.5)	Thymus: atypical hyperplasia (0/43, 3/46, 7/44, 7/42) Testis: germinal epithelial degeneration (1/50, 49/50, 50/50, 47/48)	Thymus: atypical hyperplasia (0/48, 7/44, 6/49, 5/45) Ovary: hyperplasia (4/50, 11/49, 10/50, 17/50)
Neoplastic effects	Adrenal medulla: benign pheochromocytoma (17/50, 34/50, 34/50, 34/50) Kidney: renal tubule adenoma (standard evaluation - 0/50, 4/50, 2/50, 6/50; extended evaluation - 1/50, 7/50, 15/50, 11/50; standard and extended evaluations combined - 1/50, 10/50, 15/50, 15/50); renal tubule adenoma or carcinoma (standard evaluation - 0/50, 5/50, 3/50, 7/50; extended evaluation - 1/50, 7/50, 15/50, 11/50; standard and extended evaluations combined - 1/50, 10/50, 16/50, 16/50)	Adrenal medulla: benign pheochromocytoma (3/50, 11/50, 9/50, 2/49); benign or malignant pheochromocytoma (3/50, 12/50, 10/50, 2/49)	All organs: histiocytic sarcoma (1/50, 3/50, 11/50, 12/49); malignant lymphoma (thymic origin) (0/50, 4/50, 7/50, 2/49)	All organs: histiocytic sarcoma (0/50, 2/50, 7/50, 7/50); malignant lymphoma (all types) (15/50, 28/50, 33/50, 25/50); malignant lymphoma (thymic origin) (1/50, 9/50, 10/50, 7/50) Ovary: benign sex-cord stromal tumor (0/50, 7/49, 6/50, 5/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Phenolphthalein (continued)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Decreased incidences	None	None	Liver: hepatocellular adenoma (22/50, 12/50, 8/50, 10/49); hepatocellular adenoma or carcinoma (27/50, 20/50, 11/50, 16/49); clear cell focus (24/50, 6/50, 1/50, 0/49); eosinophilic focus (22/50, 6/50, 1/50, 1/49); mixed cell focus (6/50, 2/50, 1/50, 0/49)	Liver: hepatocellular adenoma (17/50, 2/50, 6/50, 1/50); hepatocellular adenoma or carcinoma (21/50, 3/50, 6/50, 2/50); eosinophilic focus (20/50, 4/50, 2/50, 1/50)
Level of evidence of carcinogenic activity	Clear evidence	Some evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:			Negative in strains TA98, TA100, TA1535, and TA1537 with and without S9	
Sister chromatid exchanges				
Cultured Chinese hamster ovary cells *in vitro*:			Negative with and without S9	
Chromosomal aberrations				
Cultured Chinese hamster ovary cells *in vitro*:			Positive with S9; negative without S9	
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:			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.

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on phenolphthalein on December 5, 1995, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On December 5, 1995, the draft Technical Report on the toxicology and carcinogenesis studies of phenolphthalein received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of phenolphthalein by describing the uses of the chemical and rationale for the study, describing the experimental design, reporting on survival and body weight effects, and commenting on chemical-related neoplasms and nonneoplastic lesions in male and female rats and mice. Dr. Dunnick noted that molecular biology studies in collaboration with NIEHS intramural scientists are in progress or planned, including studies of the chemical in transgenic mouse models. Pharmacokinetic studies are in progress. Dr. J.R. Hailey, NIEHS, presented photomicrographs of lesions of the hematopoietic system, examples of histiocytic sarcoma, malignant lymphoma of thymic origin and associated hyperplasia, ovarian proliferative lesions, and testicular atrophy. The proposed conclusions were *clear evidence of carcinogenic activity* of phenolphthalein in male F344/N rats, *some evidence of carcinogenic activity* of phenolphthalein in female F344/N rats, and *clear evidence of carcinogenic activity* in male and female B6C3F$_1$ mice.

Dr. Goldsworthy, a principal reviewer, agreed with the proposed conclusions. He stated that the Technical Report should provide assurance that biological responses observed, especially those related to estrogenic effects, are attributable to phenolphthalein and not to lipophilic impurities. Small quantities of such impurities have been shown to account for the estrogenic activity in commercial preparations of the sulfonated analog of phenolphthalein, phenolsulfonphthalein or phenol red. Dr. Dunnick responded that the phenolphthalein used in these studies was 99% pure. She noted that Dr. M.D. Shelby, NIEHS, is developing assays for estrogenic activity and anticipated that the estrogenic potential of phenolphthalein could be compared with those of phenol red and of lipophilic impurities. Dr. Goldsworthy said there should be a comprehensive treatment of the estrogenic responses; for example, discussion on decreased incidences of liver lesions in mice. Dr. Dunnick proposed isolating the 1% impurity and examining its carcinogenic and estrogenic activity in short-term model systems such as transgenics and MCF-7 cells.

Dr. Ward, the second principal reviewer, was unable to attend the meeting but had submitted his review, which Dr. L.G. Hart, NIEHS, read into the record. Dr. Ward agreed with the proposed conclusions. He asked if the myelofibrosis reported was the typical lesion found in aging B6C3F$_1$ mice and probably estrogenic in origin. Dr. Hailey said that it was the lesion associated with aging.

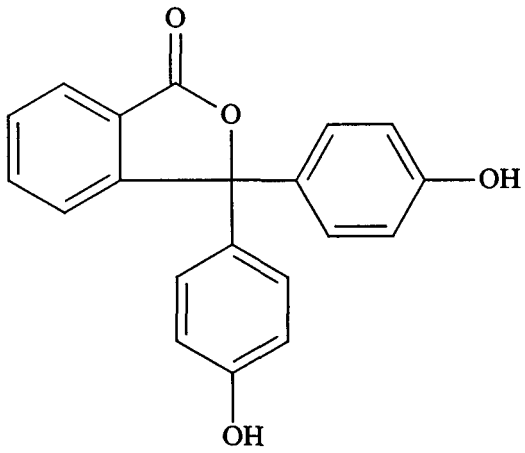
Dr. Taylor, the third principal reviewer, agreed with the proposed conclusions. He noted that pharmacokinetic data on phenolphthalein are rare in the literature and recommended that the pharmacokinetic data, particularly the lack of a dose-response relationship, in Appendix O be discussed in the body of the Technical Report. Dr. Dunnick said that these studies were limited, but that she would try to give more emphasis to the findings. Dr. Goldsworthy hoped that follow-up studies would look at phenolphthalein levels in organs such as the ovaries. Dr. G.W. Lucier, NIEHS, reported that follow-up pharmacokinetic studies will span a very wide dose range and will enable a better definition of the leveling off of tissue or blood levels of phenolphthalein in relation to dose.

Dr. Russo commented that there did not seem to be any influence of chemical treatment on pituitary gland or mammary gland lesions, with an even lower incidence of thyroid gland C-cell lesions than in control animals, all speaking against an estrogenic effect. Dr. Dunnick agreed, noting that another chemical with estrogenic activity, zearalenone, had not produced increases in mammary gland lesions in rats or mice. Dr. LeBoeuf asked whether the *in vitro* increases in chromosomal aberrations and *in vivo* increases in the frequency of micronucleated erythrocytes were consistent with other chemicals that may

have estrogen-like properties and can form quinones with metabolism, such as diethylstilbestrol. Dr. Shelby replied that the database is too small to reach any general conclusion. However, these increases along with the 2-year study results are fully consistent with the endocrine disrupting estromimetic compounds. Dr. W.T. Allaben, National Center for Toxicological Research and Food and Drug Administration (FDA), thanked the NTP, and particularly Dr. Dunnick, for sharing information during the study and review process and noted that the additional studies mentioned will be helpful to the FDA in its further review toward making an assessment regarding potential human risk.

Dr. Goldsworthy moved that the Technical Report on phenolphthalein be accepted with the revisions discussed and with the conclusions as written. Dr. Taylor seconded the motion, which was accepted with six yes votes and one abstention (Dr. LeBoeuf).

INTRODUCTION



PHENOLPHTHALEIN

CAS No. 77-09-8

Chemical Formula: $C_{20}H_{14}O_4$ Molecular Weight: 318.33

Synonyms: 3,3-Bis(4-hydroxyphenyl)-1(3H)-isobenzofuranone; 3,3-bis(*p*-hydroxyphenyl)phthalide; α-(*p*-hydroxyphenyl)-α-(4-oxo-2,5-cyclohexadien-1-ylidene)-*o*-toluic acid

Trade names: Agoral®, Alophen®, Colax®, Correctol®, Dialose®, Doxidan®, Espotabs®, Evac-U-Gen®, Evac-U-Lax®, Ex-Lax®, Feen-A-Mint®, FemiLax®, Kondremul®, LaxCaps®, Lax-Pills®, Medilax®, Modane®, Phenolax®, Prulet®

CHEMICAL AND PHYSICAL PROPERTIES

Phenolphthalein is a white or yellowish white, odorless, tasteless powder consisting of minute triclinic crystals (often twinned). It has a specific gravity of 1.277 at 32/4° C and a melting point range of 262° to 263° C (Weast, 1987) or 258° to 262° C (*Merck Index*, 1989), depending on the relative amounts of associated impurities. Although not flammable, phenolphthalein emits acrid smoke and fumes when heated to decomposition (*Sax's*, 1992). Phenolphthalein is readily soluble in alcohol (1 g dissolves in 12 to 15 mL) or ether (1 g dissolves in approximately 100 mL) and very slightly soluble in chloroform. It is almost insoluble in water; however, its solubility is increased in physiologic buffered solutions simulating intestinal contents, i.e., 7.8 mg/dL in Krebs-Ringer-bicarbonate solution, pH 7.4 (Sharaiha *et al.*, 1983). The solubility of aqueous phenolphthalein is also pH dependent and does not exceed 6 mg/dL without the addition of ethanol until the pH is above the physiologic range, i.e., greater than 9 (Fantus and Dyniewicz, 1937; Hubacher, 1945). Solutions containing phenolphthalein are colorless to pH 8.5 and pink to deep red above pH 9 (Figure 1). Phenolphthalein is used as a laboratory reagent and an acid-base indicator in titrations of mineral and organic acids and most alkalies (*Merck Index*, 1989).

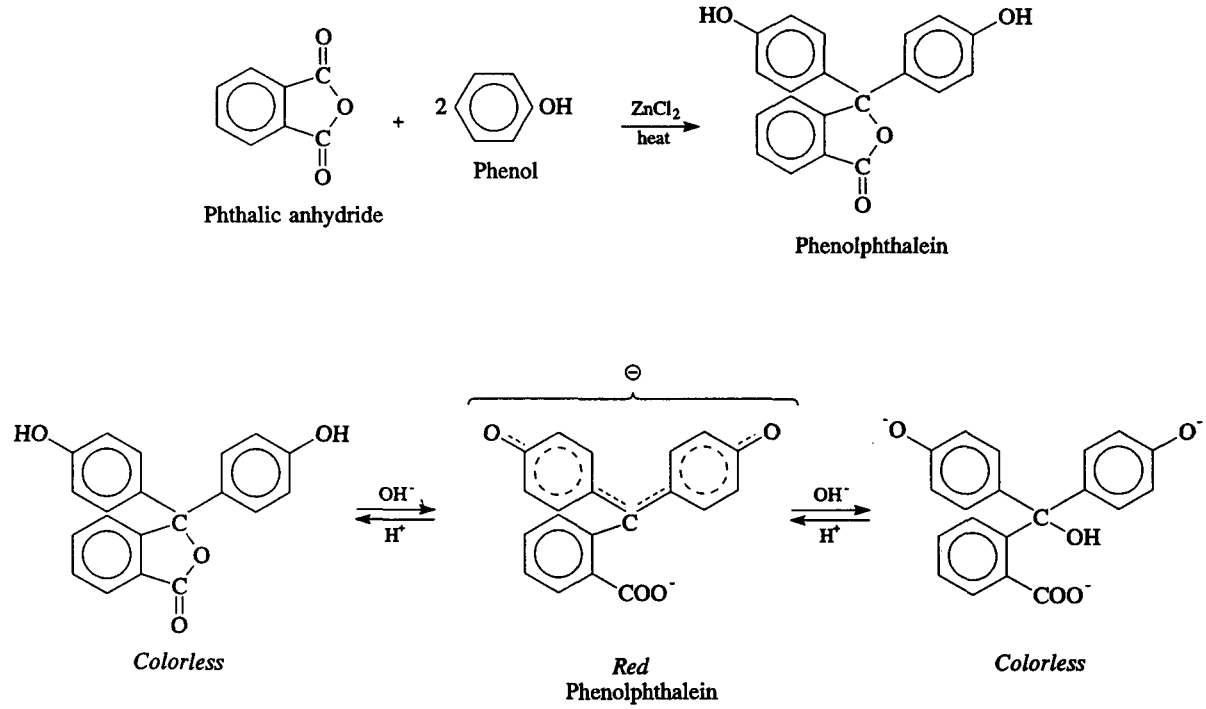


FIGURE 1
Phenolphthalein Synthesis and Reactions as an Indicator (Morrison and Boyd, 1966)

PRODUCTION, USE, AND HUMAN EXPOSURE

Phenolphthalein is synthesized commercially by the condensation of phthalic anhydride with phenol in the presence of a dehydrating agent (sulfuric acid) (Figure 1). After the mixture is heated at 120° C for 10 to 12 hours, the phenolphthalein residue is extracted with boiling water and then dissolved in dilute sodium hydroxide solution, filtered, and precipitated with acid (*Remington's*, 1990). Approximately 250 tons of phenolphthalein are produced in the United States annually by a single manufacturer, Sigma-Aldrich Corporation (*Chemical Economics Handbook*, 1992).

Early studies in humans assessing the safety of phenolphthalein when used as a pH-dependent coloring agent in adulterated wine led to recognition of its cathartic action (von Vámossy, 1908). Phenolphthalein is available worldwide in numerous proprietary over-the-counter laxative preparations including tablets, powders, suspensions, capsules, and chewables; the fact that phenolphthalein is tasteless makes it desirable for marketing in the form of chocolate candy or chewing gum (AHFS, 1995).

The usual daily oral laxative doses for white or yellow phenolphthalein are 30 to 270 mg for adults and children 12 years of age and older, 30 to 60 mg for children 6 to 11 years of age, and 15 to 30 mg for children 2 to 5 years of age, although the use of stimulant laxatives is generally not recommended in children younger than 6 years of age (*Fed. Regist.*, 1975; AHFS, 1995).

Uses of phenolphthalein as a laxative-cathartic may include: as bowel evacuants prior to bowel surgery

or radiologic, proctoscopic, and colonoscopic procedures; to alleviate pain of elimination from an episiotomy wound, anal fissures, perianal abscesses, or after surgery; to reduce intra-abdominal pressure during elimination in patients with hernias, anorectal stenosis, or abnormalities of the cerebral or coronary arterial vessels; to relieve constipation during pregnancy or the puerperium; to modify the effluent in ileostomy and colostomy patients; to compensate for lost abdominal and perineal muscle tone in geriatric patients with poor eating habits; and for altered bowel motility in patients receiving anticholinergic or narcotic therapy (Pietrusko, 1977; *Goodman and Gilman's*, 1990).

However, laxative-cathartics, which are habit forming (i.e., chronic usage results in dependence), are commonly used to self-medicate symptoms of constipation and are frequently abused in an effort to control weight (Pietrusko, 1977; *Goodman and Gilman's*, 1990). Ten percent of college students who participated in a 1981 survey admitted to purging behavior, i.e., laxative use and self-induced vomiting (Halmi *et al.*, 1981). Purging behavior is also associated with the eating disorders anorexia nervosa and bulimia nervosa; the prevalence of laxative abuse in patients with bulimia nervosa has been reported as 38% to 63% (Van Rooyen and Ziady, 1972; Pyle *et al.*, 1981; Johnson *et al.*, 1982; Bo-Linn *et al.*, 1983; Mitchell *et al.*, 1983; Fairburn and Cooper, 1984). A review by Cummings (1974) indicated that over 90% of chronic laxative abusers are women.

It is not possible to quantitate exposure to phenolphthalein via medication because of the large number of over-the-counter laxative preparations (Fleischer *et al.*, 1969; Van Rooyen and Ziady, 1972; Cummings *et al.*, 1974; LaRusso and McGill, 1975; Bytzer *et al.*, 1989). In the United States, Great Britain, and Australia, the rate of regular laxative use in populations who are apparently well is approximately 17% to 20% (Connell *et al.*, 1965; Dent *et al.*, 1986; Wu *et al.*, 1987; Kune, 1993). In 1975, 130 million dollars were spent in the United States on over-the-counter laxative preparations (Binder and Donowitz, 1975). In 1980, overall United States sales increased to approximately one billion dollars (Curry, 1990).

According to the National Occupational Exposure Survey, 75,243 workers (26% of whom were female) were potentially exposed to phenolphthalein in the years 1981 to 1983. Of the potentially exposed workers, 20,122 (65% female) were employed in the health services (NIOSH, 1990).

Regulatory Status

Based on a review of over-the-counter laxative-cathartics conducted by a Food and Drug Administration panel as part of a drug efficacy study implementation project, phenolphthalein was approved for human use and considered to be "generally recognized as safe" (*Fed. Regist.*, 1975).

Pharmacokinetics, Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Phenolphthalein is absorbed from the intestine and is excreted in the bile, urine, feces, and milk (Visek *et al.*, 1956; AHFS, 1995). Once phenolphthalein is absorbed, it is conjugated with glucuronic acid via uridine diphosphate glucuronosyltransferase (UDPGT; a phospholipid-dependent enzyme system) in the liver and intestine (Sund and Hillestad, 1982) and is distributed throughout the body in the blood and lymph (Visek *et al.*, 1956). Studies reported in the literature provide evidence for enzyme multiplicity of UDPGT. Steroidal and nonsteroidal UDPGT may have different membrane environments, and certain tissues such as the kidney and uterus have been shown to have a lower UDPGT activity for xenobiotic estrogenic compounds such as phenolphthalein (Lucier and McDaniel, 1977). Minor amounts of sulfate conjugate metabolites have also been detected in mucosal sheets isolated from the jejunum and colon of the guinea pig (Sund and Lauterbach, 1986).

Phenolphthalein conjugation enzyme activity (uridine diphosphatase glucuronosyltransferase) is absent or very low in microsomes from fetal or neonatal rat and guinea pig liver compared to activity from adult livers (Jondorf *et al.*, 1958; Wishart, 1978a). Glucuronide conjugation activity in adult rats is

stimulated by phenobarbital and unaffected by 3-methylcholanthrene (Wishart, 1978b).

Pharmacokinetic and tissue distribution studies in mice (strain not specified) and dogs (breed not specified) given 4.8 mg [^{14}C]-phenolphthalein/kg (uniformly labeled) indicated that phenolphthalein was widely and evenly distributed throughout the body, with levels of radioactivity parallel to the concentration in the blood. In dogs, approximately 50% of an oral dose was recovered in the feces and 36% in the urine after 72 hours (Visek *et al.*, 1956).

In the mouse studies (Visek *et al.*, 1956), no respiratory [^{14}C]-carbon dioxide was recovered following administration of phenolphthalein labeled with ^{14}C on the nonaromatic carbon of the lactone ring, indicating that the bonds to the labeled carbon atoms probably were not broken. Within 48 hours approximately 96% of the administered radioactivity was recovered in the urine and feces (56% in the urine and 38% in the feces following an oral dose; 30% in the urine and 68% in the feces following an intravenous dose). At 30 minutes to 6 hours, the highest levels of radioactivity appeared in the liver, gallbladder, and small intestine. On a total organ basis, the small intestine, which plays a major role in the excretion of phenolphthalein, contained more radioactive label than did the large intestine at all measurement intervals. However, when equated on a unit weight basis, the radioactivity of the large intestine and its contents increased substantially 6 hours after dosing.

Whole-body autoradiography studies in male BOM:NMRI mice given an intragastric dose of 1 mL/kg [^{14}C]-phenolphthalein (10 μCi/100 g) showed initial high levels of radioactivity in the stomach, gallbladder, and small intestine at 10 and 20 minutes. Radioactivity was observed in peripheral organs (including the kidney, liver, and skin) showing that the drug was absorbed from the gastrointestinal tract. The radiolabel moved along the intestinal tract, reaching the large intestine after 2 hours and showing maximum activity in the rectum after 4 hours. No radiolabel was detected in autoradiograms 2 days after dosing (Sund *et al.*, 1986).

Phenolphthalein undergoes extensive first pass metabolism in the intestinal epithelium and liver, which results in almost complete conversion of phenolphthalein to its glucuronide (Parker *et al.*, 1980). Studies by Colburn *et al.* (1979) demonstrated that 6 hours after intravenous administration of [^{3}H]-phenolphthalein to female Wistar rats, all radioactivity in the systemic circulation was present as the conjugate. In addition, a secondary peak in blood radioactivity occurred 5 to 6 hours after intravenous administration and coincided with the absorption of [^{3}H]-phenolphthalein from the intestine following bacterial ß-glucuronidase hydrolysis of [^{3}H]-phenolphthalein glucuronide excreted in the bile. Hydrolysis of phenolphthalein glucuronide to the aglycone is a rate-limiting step in enterohepatic recirculation (Bergan *et al.*, 1982); pretreatment with antibiotics to suppress intestinal microflora decreased the absorption of [^{3}H]-phenolphthalein from 85% to 22% in female Wistar rats following intraduodenal administration of [^{3}H]-phenolphthalein glucuronide (Parker *et al.*, 1980).

In a study with beagle dogs, two males were given 8- or 10-hour intravenous infusions of phenolphthalein at a rate of 177 μg/minute per kg body weight, and food was introduced at 2, 4, 6, and 8 hours during the study to stimulate gallbladder contraction. Steady-state serum levels were 7 μg/mL for phenolphthalein and 30 μg/mL for phenolphthalein glucuronide, and significant secondary peaks occurred for both compounds, an observation consistent with enterohepatic recirculation (Wilhelm *et al.*, 1992).

Phenolphthalein has been used as a model compound for enterohepatic recirculation studies in rats (Colburn *et al.*, 1979), and surgical cannulation of the bile duct has been used to evaluate the extent of enterohepatic recirculation in rats and dogs. In bile-duct-cannulated female Wistar rats, 95% of an intraperitoneal dose of 25 mg [^{3}H]-phenolphthalein/kg was recovered in the bile as the glucuronide within 24 hours, while only 0.2% was recovered in the urine. Administration of the same dose to intact rats resulted in recovery of 86% in the feces, predominantly as the parent drug, and 10% in the urine as the glucuronide (Parker *et al.*, 1980). In another study in female Wistar rats with biliary fistulae, phenolphthalein was completely eliminated in the bile (100%), almost entirely in the form of phenolphthalein glucuronide (98%) (Millburn *et al.*, 1967). The

plasma disappearance and biliary excretion kinetics of phenolphthalein glucuronide in the rat have been characterized by Mehendale (1990). Male Sprague-Dawley rats of the CR-1 strain were intravenously administered 3, 30, or 60 mg phenolphthalein or 3, 30, or 100 mg phenolphthalein glucuronide, and the femoral vein, artery, and common bile duct were cannulated. After administration of phenolphthalein, 99.5% of the dose was eliminated in the bile as phenolphthalein glucuronide with only trace quantities (0.5%) as phenolphthalein. Following administration of phenolphthalein glucuronide, phenolphthalein was undetectable in the bile. Biliary excretion was saturable at higher doses of both compounds.

Female dogs (breed not specified) given 4.8 mg [^{14}C]-phenolphthalein/kg excreted 51% and 36% of the administered radioactivity in the feces and urine within 72 hours after an oral dose and 54% and 37% in the feces and urine following an intravenous dose. Following surgical cannulation of the bile duct, recovery of orally administered radiolabel from the same dogs over a 72-hour period was: feces, 31%; urine, 38%; and bile, 22%. The corresponding figures following an intravenous dose were: feces, 11%; urine, 35%; and bile, 43% (Visek *et al.*, 1956).

The investigations of Visek *et al.* (1956) also demonstrated that radiolabeled phenolphthalein crossed the placenta of mice, resulting in recovery of radiolabel from fetal tissues at 6, 24, and 96 hours after administration that paralleled that of maternal blood. In the dog, however, analysis of blood and tissue samples of puppies born 50 hours after the mother received a 4.8 mg/kg oral dose indicated less than 0.03% of the administered dose in the liver and gallbladder and no radiolabel in the blood, which was interpreted by the authors as exceedingly limited passage of the drug across the placenta.

Humans

Phenolphthalein absorption in humans has been estimated to be 15% of an oral dose (*Goodman and Gilman's*, 1990). The absorbed compound is excreted primarily in the urine as phenolic-hydroxyglucuronide or sulfate conjugates, and the urine becomes pink or red if it is sufficiently alkaline. Some conjugated compound is also excreted in the feces via the bile, and the resulting enterohepatic recirculation probably contributes to prolongation of the laxative effect, a hypothesis supported by the observation that phenolphthalein is ineffective as a laxative in jaundiced patients or experimental animals with ligated common bile ducts (Steigmann *et al.*, 1938; *Goodman and Gilman's*, 1990).

Small doses of phenolphthalein in humans (30 to 60 mg) are excreted entirely as conjugated metabolites in urine or feces, while larger doses (300 mg) result in excretion of both the free and conjugated drug (Williams, 1959).

Diarrhea in infants may be caused by phenolphthalein usage by the mother during breast feeding (Tyson *et al.*, 1937).

PHARMACOLOGY

Following oral administration of phenolphthalein, evacuation usually occurs within 4 to 8 hours, and a single dose may produce laxation for several days (AHFS, 1995). The major site of cathartic action for phenolphthalein in humans is considered to be the large intestine with minor changes occurring in the small intestine (Saunders *et al.*, 1978; Sund, 1983). Phenolphthalein has choleretic properties and has been shown to accelerate bile secretions in male rats (Takeda and Aburada, 1981).

Phenolphthalein, a diphenylmethane derivative, belongs to the class of stimulant or irritant laxative-cathartics originally thought to stimulate peristalsis by irritation of the colonic mucosa (Binder, 1977). Current views of the mechanism of action of phenolphthalein indicate that it has a hydrophoric effect, which alters intestinal fluid and electrolyte movement and causes a net accumulation of luminal fluid and increased fluidity of the intestinal contents (Binder and Donowitz, 1975; Gaginella and Bass, 1978; Sund, 1983). This effect on the sodium pump, which has been demonstrated in an isolated rabbit ileal loop model *in vivo* using measurement of ^{24}Na flux, involves inhibition of Na^+ ion transport from the gut lumen to the plasma. Accumulation of Na^+ ions in the lumen results in secondary accumulation of anions (chiefly Cl^- and HCO_3^-) to maintain electroneutrality at a pH of approximately 8 with luminal water retention (Phillips *et al.*, 1965). Inhibition of intestinal water absorption has been demonstrated in

experimental animal models and in humans. An *in vivo* model using isolated jejunal, ileal, and colonic segments of the intestine of male Sprague-Dawley rats was used to demonstrate that phenolphthalein inhibited net water transport to the same extent in all regions of the intestine (Saunders *et al.*, 1978). A study of six ileostomy patients by the same investigators indicated that inhibition of water absorption in both the large and small intestine contributes to the laxative effect of phenolphthalein; 100 mg given four times a day increased the weight of ileostomy output by 30% and Na^+ output by 39%.

In addition to inhibition of active transport of sodium across the rabbit ileum and frog skin (Phillips *et al.*, 1965), phenolphthalein inhibited the sodium-dependent uptake of 3-methyl-D-glucose by the hamster small intestine *in vitro* (Adamič and Bihler, 1967) and reduced intestinal glucose absorption in the rat (Hart and McColl, 1967). Chignell (1968) investigated the effects of various purgative drugs on Na^+, K^+-adenosine triphosphatase activity in microsomes from the intestinal brush border of male Sprague-Dawley rats. Phenolphthalein was the most potent enzyme inhibitor of all the purgatives studied, blocking 70% of enzyme activity, which suggests that inhibition of sodium transport is a direct result of inhibition of Na^+, K^+-adenosine triphosphatase.

Phenolphthalein has been shown to stimulate prostaglandin biosynthesis in the colon of female Sprague-Dawley rats, with release of E-series prostaglandins into the gut lumen (Cohen, 1982). The extent of prostaglandin release correlated with the net water flux (laxative action) induced by the drug, and pretreatment with inhibitors of prostaglandin synthesis (indomethacin or aspirin) reduced the laxative effect in rats and mice (Beubler and Juan, 1978; Capasso *et al.*, 1984). In *in vitro* studies in male rat intestinal homogenates, phenolphthalein (314 μM) significantly increased the conversion of arachidonic acid, a prostaglandin precursor, to prostaglandins and 5-hydroxy-eicosatetraynoic acid in the colon and to leukotriene B_4 in the jejunum and colon. Similarly, in human colon homogenates, 100 μg phenolphthalein/mL increased conversion of arachidonic acid to prostaglandins and leukotriene B_4 (Capasso *et al.*, 1987).

Autore *et al.* (1984) attributed the laxative effect of a 16 mg/kg intragastric dose of phenolphthalein in male Wistar rats to stimulation of histamine and 5-hydroxytryptamine production and increased formation of prostaglandins, effects which were reduced by pretreatment with indomethacin. They concluded that the stimulation of biologically active amines may be due to altered gut motility, an overproduction of prostaglandin-like material, or a non-related direct effect of phenolphthalein. In a more recent study, there was an increase in kininogen content (measured as bradykinin equivalents/g wet tissue weight) in the colon of phenolphthalein-treated male Wistar rats, thus implicating kinins in the induction of laxation (Autore *et al.*, 1990). Augmentation of muscle contractions induced by prostaglandin E_2, demonstrated in the rat stomach and the longitudinal muscle of the guinea pig ileum and colon following *in vitro* administration of 10 μg phenolphthalein/mL, was suggested as a possible contributing factor in phenolphthalein laxation (Capasso *et al.*, 1988).

Although the laxative effect of phenolphthalein in monkeys is similar to that in humans, the dose required to induce laxation (25 mg/kg) is approximately 10 times that required for humans (Loewe and Hubacher, 1941).

TOXICITY

Experimental Animals

Phenolphthalein, given intravenously in dogs (breed not specified), was classified as relatively nontoxic in early studies (Abel and Rowntree, 1909). However, in rats it is regarded as moderately toxic by the intraperitoneal route, with an LD_{Lo} of 500 mg/kg (*Sax's*, 1992).

In oral studies, female mice (strain not specified) fed 5, 25, or 50 mg phenolphthalein/kg per day for 135 days showed no toxic manifestations or evidence of histopathologic changes in the liver, kidney, or gastrointestinal tract (Visek *et al.*, 1956).

Phenolphthalein, at doses of 25 and 50 μg/mL, caused cytotoxic effects in cultured Chang liver cells

characterized by decreased cell growth and increased anaerobic glycolysis, i.e., increased glucose consumption and lactate production (Nishikawa, 1981). Phenolphthalein has also been shown to inhibit growth of strains of anaerobic bacteria *in vitro* and cause leakage of potassium ions from both anaerobes and aerobes, findings consistent with the antibacterial properties of phenolic compounds (Bergan *et al.*, 1982; Sund, 1983).

Humans

Under normal conditions, phenolphthalein is regarded as nontoxic and safe for consumption, although therapeutic oral doses may occasionally produce abdominal discomfort, diarrhea, nausea, decreased blood pressure, faintness, and red urine and feces (AHFS, 1995). Serious side effects have been reported in cases of habitual phenolphthalein consumption under conditions of abuse (Cooke, 1977; Pietrusko, 1977); hypersensitivity reactions have been limited to susceptible or allergic individuals (Davies, 1985).

The primary reported organ for phenolphthalein toxicity is the intestine; indiscriminate use of phenolphthalein results in chronic constipation and laxative dependence, loss of normal bowel function, and bowel irritation. Habitual use for several years may cause a "cathartic colon," i.e., a poorly functioning, atonic dilation of the colon, especially of the right side, resulting in extensive bowel retention. The clinical condition, which resembles chronic ulcerative colitis both radiologically and pathologically, involves thinning of the intestinal wall and loss of the normal mucosal pattern of the terminal ileum (Cummings, 1974; Cooke, 1977; Pietrusko, 1977; AHFS, 1995).

Anecdotal cases of long-term use or overdose of phenolphthalein have been associated with abdominal pain, diarrhea, vomiting, electrolyte imbalance (hypokalemia, hypocalcemia, and/or metabolic acidosis or alkalosis), dehydration, malabsorption, protein-losing gastroenteropathy, steatorrhea, anorexia, weight loss, polydipsia, polyuria, cardiac arrhythmias, muscle weakness, prostration, and histopathologic lesions (Heizer *et al.*, 1968; Velentzas and Ikkos, 1971; Cummings, 1974; LaRusso and McGill, 1975; Pohl and Lowe, 1978; AHFS, 1995). Kidney, muscle, and central nervous system disturbances are thought to be due to electrolyte imbalance. Intestinal sodium and water loss stimulates compensatory renin production and secondary aldosteronism, leading to sodium conservation and potassium loss by the kidney (hypokalemia). Hypokalemia contributes to renal insufficiency, sometimes associated with rhabdomyolysis (Copeland, 1994).

Abuse of phenolphthalein-containing laxatives has been associated with gastrointestinal bleeding and iron deficient anemia (Weiss and Wood, 1982), acute pancreatitis (Lambrianides and Rosin, 1984), and multiple organ damage in cases of massive overdosages, including fulminant hepatic failure and disseminated intravascular coagulation (Sidhu *et al.*, 1989). Individual hypersensitivity reactions to phenolphthalein have also been reported to cause renal irritation, encephalitis, cardiac arrest, respiratory disturbances, and death (AHFS, 1995).

Phenolphthalein allergy is often manifested by cutaneous inflammatory reactions or fixed drug eruptions, i.e., solitary or multiple, well-defined, erythematous macules that may progress to vesicles and/or bullae. These lesions characteristically recur in the same location with each subsequent dose of phenolphthalein and generally leave residual hyperpigmentation that increases in intensity with each exposure; numerous melanin-containing dermal macrophages have been demonstrated in pigmented areas (Wyatt *et al.*, 1972; Davies, 1985; Stroud and Rosio, 1987; Zanolli *et al.*, 1993). In extreme cases, recurrences have involved progressively more severe lesions characterized as bullous erythema multiforme, with focal hemorrhage and necrosis and perivascular lymphocytic infiltration (Shelley *et al.*, 1972), and in one case report, toxic epidermal necrolysis (Kar *et al.*, 1986).

A review of 204 cases of phenolphthalein ingestion in children 5 years of age and younger reported to the Pittsburgh Poison Center over a 30-month period indicated that ingestion of 1 g or less was associated with minimal risk of developing dehydration caused by excessive diarrhea and resulting fluid loss (Mrvos *et al.*, 1991). However, despite the low acute toxicity profile documented by the Pittsburgh study, cases of fatal phenolphthalein poisoning of children have been reported; symptoms of pulmonary and

cerebral edema, multiple organ toxicity, and encephalitis were attributed to hypersensitivity reactions (Cleves, 1932; Kendall, 1954; Sarcinelli *et al.*, 1970). Repeated administration of phenolphthalein-containing laxatives to children has led to serious illnesses and multiple hospitalizations (Fleisher and Ament, 1977; Meadow, 1977; Devore *et al.*, 1982; Rosenberg, 1987; Sugar *et al.*, 1991; Ayass *et al.*, 1993).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

In a three-generation study involving 678 births, no drug-related teratogenic or reproductive deficits were observed in mice (strain not specified) fed approximately 250 mg phenolphthalein/kg per day in chocolate (Stockinger, 1965).

Phenolphthalein has weak estrogenic activity. In a study in immature Wistar rats, the minimum effective dose of phenolphthalein required to cause an estrogenic response (increase in glycogen content) in the uterus was 4 mg, compared to a 0.1 mg minimum effective dose of diethylstilbestrol (Bitman and Cecil, 1970). Subcutaneous injection of phenolphthalein also stimulated growth of the immature rat uterus in a similar study (Nieto *et al.*, 1990).

Humans

Phenolphthalein competed with estrogen for binding to cultured MCF-7 human breast cancer cells, with a relative binding affinity 10^{-4} that of estradiol, and stimulated cell growth as measured by DNA and protein assays. Acting as an estrogen agonist, phenolphthalein induced elevated levels of progesterone receptor in the MCF-7 cells. Growth stimulation by both phenolphthalein and estradiol was blocked by the anti-estrogen, 4-hydroxytamoxifen (Ravdin *et al.*, 1987). No other information related to the reproductive or developmental toxicity of phenolphthalein in humans has been reported in the literature.

CARCINOGENICITY

Experimental Animals

No definitive information regarding the carcinogenic potential of phenolphthalein in experimental animals was found in the literature. However, phenolphthalein phosphate was inactive as an inducer of tumors at the site of subcutaneous injections in mice when given once weekly for 13 weeks. Following an 8-month, treatment-free period, no tumors were observed when the experiment was terminated at 11 months with a 50% survival rate (10/20). The value of this study as an indicator of the carcinogenic potential of phenolphthalein was limited because the compound was tested at a single unspecified dose level (Haddow and Horning, 1960).

Humans

In a study conducted in Melbourne, Australia with 1,408 subjects, there was no statistically significant increased risk for colorectal cancer in phenolphthalein laxative users (Kune, 1993). The cases included in this study included all histologically confirmed new patients in "The Melbourne Colorectal Cancer Study" from April 1980 to April 1981. Community controls were age/sex frequency-matched with the clinical cases and were randomly selected from the same geographic area. In another study following 11,888 residents of a retirement community in California for 4.5 years, the association between laxative use and risk of colorectal cancer was not significant (Wu *et al.*, 1987). Information on other cancer sites was not reported.

GENETIC TOXICITY

The mutagenicity of phenolphthalein has been investigated and the data indicate that the chemical, although not mutagenic in bacteria, is capable of inducing chromosomal damage in mammalian cells *in vitro* and *in vivo*. Phenolphthalein did not induce DNA damage in repair-deficient strains of *Bacillus subtilis* (Kada *et al.*, 1972; Fujita *et al.*, 1976), nor was it mutagenic in *Salmonella typhimurium* (Bonin *et al.*, 1981; Mortelmans *et al.*, 1986). However,

blood samples obtained from the mice at the end of the 13-week toxicity study with phenolphthalein showed significant increases in micronucleated polychromatic erythrocytes and normochromatic erythrocytes in males and females (Dietz *et al.*, 1992). Efforts to confirm the genotoxicity of phenolphthalein in mice resulted in a series of experiments that investigated the influence of various experimental parameters (route of administration, frequency of dosing, duration of exposure, carrier vehicle) on the phenolphthalein-induced micronucleus response in mice (Witt *et al.*, 1995). It was concluded that phenolphthalein at relatively high doses (greater than or equal to 2,000 mg/kg per day for at least 2 days) administered either in feed or by gavage induced micronuclei in erythrocytes of $B6C3F_1$ mice and that this indication of chromosomal damage could be detected in either bone marrow or blood. Lower doses of phenolphthalein (120 mg/kg per day) were highly effective in inducing micronucleated erythrocytes when administered over a longer period of time (14 weeks) in Swiss (CD-1®) mice. Phenolphthalein also induced dose-related increases in chromosomal aberrations in cultured Chinese hamster ovary cells treated in the presence of induced rat liver S9 (Witt *et al.*, 1995). These *in vitro* results provide additional evidence of the genotoxicity of phenolphthalein.

Study Rationale

The National Cancer Institute nominated phenolphthalein for study because of its widespread use as a component of numerous over-the-counter laxative preparations, and the lack of adequate testing for carcinogenicity in experimental animals.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF PHENOLPHTHALEIN

Phenolphthalein was obtained from Air Products and Chemicals, Inc. (Allentown, PA), in one lot (127-7809) and from Pharmco Laboratories, Inc. (Titusville, FL), in two lots (P3186-D5 and P9189-J1). Lot 127-7809 was used during the 14-day studies, lot P3186-D5 was used during the 13-week studies, and lot P9189-J1 was used during the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix J). Reports on analyses performed in support of the phenolphthalein studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

All lots of the chemical, a yellow powder, were identified as phenolphthalein by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy and by melting point. The purity of all lots was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography, and high-performance liquid chromatography. For lot 127-7809, elemental analyses for carbon, hydrogen, and oxygen were in agreement with the theoretical values for phenolphthalein. Karl Fischer water analysis indicated 0.36% ± 0.02% water. Functional group titration indicated a purity of 98.8% ± 0.8%. Thin-layer chromatography by two systems indicated a major spot, a minor impurity, a trace impurity, and a slight trace impurity. High-performance liquid chromatography with one system revealed a major peak and five impurities with a combined area of 2.19% relative to the major peak area; high-performance liquid chromatography with a second system revealed a major peak and four impurities with a combined area of 2.28% relative to the major peak area. The overall purity of lot 127-7809 was determined to be greater than or equal to 98%. For lot P3186-D5, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for phenolphthalein. Karl Fischer water analysis indicated 0.193% ± 0.004% water. Functional group titration indicated a purity of 99.5% ± 0.4%. Thin-layer chromatography indicated a major spot, a minor impurity, and a trace impurity by one system and a major spot and a trace impurity by a second system. High-performance liquid chromatography revealed a major peak and three impurities with a combined area of 1.4% relative to the major peak area, and major peak comparisons of lot P3186-D5 with lot 127-7809 indicated a purity of 100.3% ± 0.6% for lot P3186-D5 relative to lot 127-7809. The overall purity of lot P3186-D5 was determined to be greater than or equal to 98%. For lot P9189-J1, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for phenolphthalein. Karl Fischer water analysis indicated 0.12% ± 0.05% water. Functional group titration indicated a purity of 99.9% ± 0.5%. Thin-layer chromatography indicated a major spot and a trace impurity by one system and a major spot by a second system. High-performance liquid chromatography revealed a major peak and three impurities with a combined area of 1.2% relative to the major peak area, and major peak comparisons of lot P9189-J1 with lot 127-7809 indicated a purity of 100.9% ± 0.2% for lot P9189-J1 relative to lot 127-7809. The overall purity of lot P9189-J1 was determined to be greater than or equal to 99%.

Stability studies of lot 127-7809 were performed by the analytical chemistry laboratory using high-performance liquid chromatography. These studies indicated that phenolphthalein was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored protected from light at 25° C in sealed containers during the 13-week studies and was stored protected from light at or below 27° C in sealed containers during the 2-year studies.

Stability was monitored during the 13-week and 2-year studies using high-performance liquid chromatography. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations for all studies were prepared weekly by mixing phenolphthalein with feed (Table J1). Stability studies of the 6,000 ppm dose formulation of lot 127-7809 were performed by the analytical chemistry laboratory using high-performance liquid chromatography. The stability of the dose formulation was confirmed for at least 2 weeks when stored at temperatures up to 25° C. Homogeneity and stability studies of the 500 ppm dose formulation of lot P3186-D5 were performed by the analytical chemistry laboratory using high-performance liquid chromatography. Homogeneity was confirmed and the stability of the dose formulation was confirmed for at least 3 weeks when stored protected from light at room temperature.

Periodic analyses of the dose formulations of phenolphthalein were conducted at the study laboratories using high-performance liquid chromatography. During the 13-week studies, the formulations were analyzed every 6 weeks (Table J2). During the 2-year studies, the formulations were analyzed approximately every 8 weeks (Table J3). All of the dose formulations analyzed during the 13-week studies were within 10% of the target concentrations. Of the dose formulations analyzed during the 2-year studies, 99% (122/123) were within 10% of the target concentrations; the one formulation out of specifications was remixed and was within 10% of the target concentration. During the 2-year studies, 89% (40/45) of the animal room samples were within 10% of the target concentrations. Results of periodic referee analyses performed during the 13-week studies by the analytical chemistry laboratory agreed with the results obtained by the study laboratory (Table J4).

14-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries (Indianapolis, IN), and animals were quarantined for 19 days. Before initiation of the studies, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Groups of five male and five female rats and mice were fed diets containing 0, 6,250, 12,500, 25,000, 50,000, or 100,000 ppm phenolphthalein. Feed and water were available *ad libitum*. Rats and mice were housed five per cage. Clinical findings were recorded daily for rats and mice. Feed consumption was recorded on days 7 and 14 and whenever the feed had to be replenished due to an inadequate feed supply. The animals were weighed initially, on day 7, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 14-day studies, a necropsy was performed on all rats and mice. Histopathologic examinations were performed on one male control rat, two female control rats, two male control mice, one female control mouse, and two males and one female from the 100,000 ppm rats and mice. Table 1 lists the tissues and organs examined.

13-WEEK STUDIES

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to phenolphthalein and to determine the appropriate exposures to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 12 to 14 days (rats) or for 14 days (mice) and were 6 weeks old on the first day of the studies. Before initiation of the studies, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female sentinel rats and on five male and five female control mice using the protocols of the NTP Sentinel Animal Program (Appendix M).

Groups of 10 male and 9 or 10 female rats and mice were fed diets containing 0, 3,000, 6,000, 12,000, 25,000, or 50,000 ppm phenolphthalein. Additional groups of 10 male and 10 female rats designated for clinical pathology evaluations were

fed diets containing 0, 3,000, 6,000, 12,000, 25,000, or 50,000 ppm phenolphthalein. Feed and water were available *ad libitum*. Rats were housed five per cage, and mice were housed individually. Clinical findings were recorded weekly for rats and mice. Feed consumption was recorded weekly. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

On days 5 and 21, all clinical pathology group rats were anesthetized with carbon dioxide, and blood was collected from the retroorbital sinus for hematology and clinical chemistry analyses. At the end of the 13-week study, all core study rats were anesthetized with carbon dioxide, and blood was collected from the retroorbital sinus for hematology and clinical chemistry analyses. Blood for hematology determinations was placed in tubes containing potassium EDTA as the anticoagulant. Hematology evaluations for hematocrit, hemoglobin, erythrocyte and reticulocyte counts, mean cell volume, mean cell hemoglobin, and leukocyte counts and differentials were performed according to standard hematology methods using a Baker 7000 (Baker Instruments, Allentown, PA) hematology analyzer. Platelet counts were performed using a Baker Model 810 (Baker Instruments, Allentown, PA) platelet analyzer. In addition, erythrocyte and platelet morphology were evaluated microscopically. For clinical chemistry analyses, samples were collected in plastic centrifuge tubes. Serum samples were analyzed using a Centrifichem 400 (Baker Instruments, Allentown, PA). The hematology and clinical chemistry parameters measured are listed in Table 1.

At the end of the 13-week studies, samples were collected from rats (core study) and mice exposed to 0, 12,000, 25,000, or 50,000 ppm phenolphthalein for sperm morphology and vaginal cytology evaluations. The parameters evaluated are listed in Table 1. Methods used were those described in NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1984a). For 7 consecutive days prior to the end of the studies, the vaginal vaults of the females were moistened with saline, if necessary, and aspirated samples of vaginal fluids and cells were transferred to slides, air dried, fixed, and stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous cells were determined to ascertain estrus cycle stage (i.e., diestrus, proestrus, estrus, or metestrus). Male animals were evaluated for sperm morphology, count, and motility. The right testis and right epididymis were isolated, weighed, and fixed and preserved for histopathology. 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 in five fields per slide by two observers. Following completion of the sperm motility estimates, each right cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution. Samples were then removed for morphology evaluation, and the remainder were heat fixed at 65° C. Sperm density was determined microscopically using a hemacytometer. Four sperm morphology slides were prepared for each animal evaluated. An aliquot of killed sperm suspension was stained in a test tube, spread on a microscope slide, coverslipped, and examined. Results of reproductive tissue evaluations and estrous cycle characterization are given in Appendix I.

A necropsy was performed on all core study rats and on all mice. The brain, heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on control and 50,000 ppm groups of rats and mice. Additionally, the liver (females), lung (males), and ovaries of rats were examined to a no effect level; the bone marrow and spleen (males) of mice were examined to a no effect level. Table 1 lists the tissues and organs routinely examined.

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats were fed diets containing 0, 12,000, 25,000, or 50,000 ppm phenolphthalein. Groups of 50 male and 50 female mice were fed diets containing 0, 3,000, 6,000, or 12,000 ppm phenolphthalein.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY) for use in the 2-year studies. Rats were quarantined for 13 days (males) or 15 days (females) before the beginning of the study. Mice were quarantined for 12 days (males) or 13 days (females) before the beginning of the study. Five male and five female rats and mice were selected for parasite evaluation and gross observation of disease. Male and female rats and female mice were approximately 7 weeks old at the beginning of the studies; male mice were approximately 6 weeks old at the beginning of the study. Serology samples were collected from up to five male and five female rats and mice for viral screening before the beginning of the study. Serology samples were collected from up to five male and five female sentinel rats at 5, 9, 12, 17, 18, and 23 months and from up to three male and three female sentinel rats and up to five male and five female 25,000 ppm rats at the end of the study. Serology samples were collected from up to five male and five female sentinel mice at 5, 12, and 18 months and from up to five male and five female 6,000 ppm mice at the end of the study. 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 five per cage and mice were housed individually. Feed and water were available *ad libitum*. Feed consumption was measured approximately every 4 weeks by cage. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix L.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks, and body weights were recorded initially, weekly for the first 13 weeks, and every 4 weeks thereafter.

On the last day of the 2-year studies, blood was collected from the retroorbital sinus of three male and three female anesthetized rats and mice from each exposed group at five time points (6 and 9 a.m. and 1, 4, and 9 p.m.) for the determination of plasma concentrations of total phenolphthalein (free and conjugated). The phenolphthalein concentration was not measured in the plasma of control animals because it was not expected to be a normal constituent of plasma and was not detected in plasma from untreated animals during development of the analytical procedure. Dosed feed was available *ad libitum* during the collection period. The plasma was stored at $-20°$ C or lower until analysis. Plasma samples were analyzed using a procedure developed during the single-dose toxicokinetic studies (Appendix O) for the analysis of concentrations of total phenolphthalein (free and conjugated). Plasma samples were treated with β-glucuronidase and sulfatase enzyme preparation. Solid-phase extraction was used to isolate phenolphthalein, and the samples were analyzed using high-performance liquid chromatography. The average plasma concentrations of total phenolphthalein and standard deviations were calculated. The logarithms of these values were plotted as a function of time. Results of analyses of plasma concentrations for total phenolphthalein at the end of the 2-year studies are given in Appendix H.

A complete necropsy and microscopic examination were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (i.e., adrenal gland, kidney, ovary), samples from each organ were examined. For the extended evaluation of renal tubule proliferative lesions, kidneys of male and

female rats were step sectioned at 1 mm intervals, and five or six additional sections were obtained from each animal. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The microscopic 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 reviewed the adrenal gland and kidney of male and female rats; the seminal vesicle and spleen of male rats; the ovary of female rats; the bone, bone marrow, liver, thymus, lymph nodes, and spleen of male and female mice; the testis of male mice; and the ovary, pituitary gland, thyroid gland, and uterus of female mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed all 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 pathologist, 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. Thus, the final diagnoses represent a consensus of quality assessment pathologists, the PWG chairperson, 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 diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes 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 as presented in Tables A1, A5, B1, B5, C1, C5, D1, and D5 are given as the number of animals bearing such lesions at a specific anatomic site and the number 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, i.e., the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidences

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated

cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if the fit of the model was not significantly enhanced. The neoplasm incidences of exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These methods include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time.

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 have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Clinical chemistry, hematology, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973). Because the 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 (Haseman *et al.*, 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects.

QUALITY ASSURANCE METHODS

The 13-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 covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. 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, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

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

The genetic toxicity studies of phenolphthalein are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure and responses of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

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

14-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
Cannon Laboratories, Inc. (Reading, PA)	Microbiological Associates, Inc. (Bethesda, MD)	TSI Mason Laboratories, Inc. (Worcester, MA)
Strain and Species		
F344/N rats $B6C3F_1$ mice	F344/N rats $B6C3F_1$ mice	F344/N rats $B6C3F_1$ mice
Animal Source		
Harlan Industries (Indianapolis, IN)	Taconic Farms (Germantown, NY)	Taconic Farms (Germantown, NY)
Time Held Before Studies		
19 days	Rats: 12 to 14 days Mice: 14 days	Rats: 13 days (males) or 15 days (females) Mice: 12 days (males) or 13 days (females)
Average Age When Studies Began		
Not available	6 weeks	7 weeks (male and female rats and female mice) or 6 weeks (male mice)
Date of First Dose		
4 June 1979	Rats: 28 April 1987 Mice: 30 April 1987	Rats: 13 March (males) or 15 March (females) 1991 Mice: 27 November (males) or 28 November (females) 1990
Duration of Dosing		
14 days	13 weeks	104 weeks (males) or 105 weeks (females)
Date of Last Dose		
17 June 1979	Rats: 28-29 July 1987 (core study) or 18 May 1987 (clinical pathology groups) Mice: 30-31 July 1987	Rats: 10 March (males) or 18-19 March (females) 1993 Mice: 24-25 November (males) or 3-4 December (females) 1992
Necropsy Dates		
Rats: 19-20 June 1979 Mice: 19 June 1979	Rats: 28-29 July 1987 Mice: 30-31 July 1987	Rats: 10 March (males) or 18-19 March (females) 1993 Mice: 24-25 November (males) or 3-4 December (females) 1992
Average Age at Necropsy		
Not available	19 weeks	110 weeks (male mice), 111 weeks (male rats), or 112 weeks (female rats and mice)
Size of Study Groups		
5 males and 5 females	10 males and 9 or 10 females	50 males and 50 females

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Phenolphthalein (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weight	Same as 14-day studies	Same as 14-day studies
Animals per Cage		
5	Rats: 5 Mice: 1	Rats: 5 Mice: 1
Method of Animal Identification		
Ear tag	Ear punch and toe clip	Tail tattoo
Diet		
NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed daily	Same as 14-day studies, changed weekly	Same as 14-day studies, changed twice weekly
Water Distribution		
Tap water (Reading municipal supply) via water bottles changed weekly, available *ad libitum*	Tap water (Bethesda municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*, changed every 2 weeks	Tap water (Worcester municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*, changed every 2 weeks
Cages		
Polycarbonate cages with stainless steel tops, changed twice per week	Polycarbonate suspended cages (Lab Products, Inc., Rochelle Park, NJ), changed twice per week	Polycarbonate, solid-bottom cages (Lab Products, Inc., Rochelle Park, NJ), changed 3 times per week (male rats), twice per week (female rats), or weekly (male and female mice)
Bedding		
Absorb-Dri® hardwood chips, changed twice per week	Beta-chips® heat-treated hardwood chips (Northeastern Products Corp., Warrensburg, NY), changed twice per week	Sani-chips® heat-treated hardwood chips (P.J. Murphy Forest Products, Montville, NJ), changed 3 times per week (male rats), twice per week (female rats), or weekly (male and female mice)
Cage Filters		
Reemay® spun-bonded polyester filter sheet (Andico, Birmingham, AL)	Spun-bonded polyester filter sheet (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Nonwoven fiber filters (Snow Filtration, Cincinnati, OH), changed every 2 weeks
Racks		
Racks changed every 2 weeks	Stainless steel racks (Lab Products, Inc., Rochelle Park, NJ), changed every 2 weeks	Same as 13-week studies

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Phenolphthalein (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Animal Room Environment		
Temperature: 22° to 24° C Relative humidity: 45% to 55% Fluorescent light: 12 hours/day Room air: 10 to 15 changes/hour	Temperature: 21° to 23° C Relative humidity: 50% to 67% Fluorescent light: 12 hours/day Room air: minimum of 10 changes/hour	Temperature: 21° to 23° C Relative humidity: 37% to 58% Fluorescent light: 12 hours/day Room air: 10 changes/hour
Doses		
0, 6,250, 12,500, 25,000, 50,000, or 100,000 ppm in feed, available *ad libitum*	0, 3,000, 6,000, 12,000, 25,000, or 50,000 ppm in feed, available *ad libitum*	Rats: 0, 12,000, 25,000, or 50,000 ppm in feed, available *ad libitum* Mice: 0, 3,000, 6,000, or 12,000 ppm in feed, available *ad libitum*
Type and Frequency of Observation		
Observed once daily; animals were weighed initially, on day 7, at the end of the studies; clinical findings were recorded daily. Feed consumption was recorded by cage on days 7 and 14 and whenever the feed had to be replenished due to an inadequate feed supply.	Observed twice daily; animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly. Feed consumption was recorded weekly by cage.	Observed twice daily; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies; clinical findings were recorded every 4 weeks. Feed consumption was recorded approximately monthly by cage.
Method of Sacrifice		
Asphyxiation with carbon dioxide	Asphyxiation with carbon dioxide	Asphyxiation with carbon dioxide
Necropsy		
Necropsy was performed on all animals.	Necropsy was performed on all core study rats and on all mice. Organs weighed were brain, heart, right kidney, liver, lung, right testis, and thymus.	Necropsy was performed on all animals.
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of rats in the clinical pathology groups on days 5 and 21 and of core study rats at the end of the study for hematology and clinical chemistry. ***Hematology:*** hematocrit, hemoglobin, erythrocyte and reticulocyte counts, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet count, and total leukocyte counts and differentials. ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids.	None

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Phenolphthalein (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Histopathology		
Complete histopathology was performed on one male rat and two female rats from the control groups, on two male mice and one female mouse from the control groups, and on two males and one female from the 100,000 ppm rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal glands, bone marrow, brain, ears (external and middle), esophagus, eyes, gallbladder (mice), heart, large intestine (colon and rectum), small intestine (duodenum, jejunum, and ileum) kidneys, larynx, liver, lungs (and mainstem bronchi), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid glands, pituitary gland, prostate gland, rib (and costochondral junction), salivary gland, skin, spinal cord (and sciatic nerve), spleen, stomach, testes (with seminal vesicle), thigh muscle, thymus, thyroid glands, trachea, urinary bladder, and uterus.	Complete histopathology was performed on 0 and 50,000 ppm rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland (rats), esophagus, femur (including marrow), heart, gallbladder (mice), large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lungs (and mainstem bronchi), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid gland, pituitary gland, preputial gland (rats), prostate gland, salivary gland, skin, spleen, stomach (including forestomach and glandular stomach), testes (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. In rats, the liver (females), lung (males), and ovaries were examined to a no effect level. In mice, the bone marrow and spleen (males) were examined to a no effect level.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal glands, brain (3 sections), clitoral gland, esophagus, femur (including marrow), heart, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidneys, liver, lung (and mainstem bronchi), lymph nodes (mandibular and mesenteric), mammary gland, nose (3 sections), ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary glands, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Morphology and Vaginal Cytology Evaluations		
None	At the end of the study, sperm samples were collected from male rats (core study) and male mice in the 0, 12,000, 25,000, and 50,000 ppm groups for sperm morphology evaluations. The parameters evaluated included sperm density, morphology, and motility. The right cauda, right epididymis, and right testis were weighed. Vaginal fluid samples were collected for up to 7 consecutive days prior to the end of the studies from female rats (core study) and female mice in the 0, 12,000, 25,000, and 50,000 ppm groups. The parameters evaluated were relative frequency of estrous stages and estrous cycle length.	None
Determinations of Total Phenolphthalein in Plasma		
None	None	On the last day, blood was taken from three animals from each exposed group at 6 and 9 a.m. and 1, 4, and 9 p.m. for determination of plasma concentrations of total phenolphthalein.

RESULTS

RATS

14-DAY STUDY

All rats survived to the end of the study (Table 2). The final mean body weights of all exposed groups of rats were similar to those of the controls. Feed consumption by 100,000 ppm males and females was greater than that by the controls; feed consumption by all other exposed groups of rats was similar to that by the controls. Dietary levels of 6,250, 12,500, 25,000, 50,000, or 100,000 ppm phenolphthalein resulted in average daily doses of approximately 500, 1,000, 2,000, 4,500, or 10,500 mg phenolphthalein/kg body weight to males and 500, 1,000, 2,000, 4,000, or 11,000 mg/kg to females. The feces of 100,000 ppm males and females were abnormally lighter in color than those of the other groups. This discoloration was observed on day 3 and continued throughout the study. There were no chemical-related gross or microscopic lesions observed in rats.

TABLE 2
Survival, Mean Body Weights, and Feed Consumption of Rats in the 14-Day Feed Study of Phenolphthalein

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)		Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final		Week 1	Week 2
Male						
0	5/5	123 ± 9	174 ± 12		12	14
6,250	5/5	128 ± 13	168 ± 13	97	11	13
12,500	5/5	125 ± 6	167 ± 10	96	11	13
25,000	5/5	125 ± 8	167 ± 11	96	11	13
50,000	5/5	127 ± 11	169 ± 10	97	12	15
100,000	5/5	127 ± 13	167 ± 12	96	13	18
Female						
0	5/5	109 ± 4	136 ± 4		10	10
6,250	5/5	110 ± 6	136 ± 8	100	9	10
12,500	5/5	110 ± 6	133 ± 6	98	10	10
25,000	5/5	108 ± 5	130 ± 6	96	9	9
50,000	5/5	111 ± 7	135 ± 7	99	10	10
100,000	5/5	111 ± 6	132 ± 5	97	12	15

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

13-WEEK STUDY

All rats survived to the end of the study (Table 3). The final mean body weight of the 50,000 ppm females and the body weight gains of the 25,000 and 50,000 ppm females were significantly lower than those of the controls. The final mean body weights and body weight gains of all other exposed groups were similar to those of the controls. Feed consumption by exposed groups was similar to that by the controls. Dietary levels of 3,000, 6,000, 12,000, 25,000, or 50,000 ppm phenolphthalein resulted in average daily doses of approximately 200, 400, 800, 1,600, or 3,500 mg phenolphthalein/kg body weight to males and 200, 400, 800, 1,700, or 3,600 mg/kg to females. There was no cathartic action or any other clinical finding attributed to exposure to phenolphthalein.

The few differences in the hematology and clinical chemistry parameters were sporadic and were not considered to be chemical related (Table G1).

The percentage of motile sperm in the 12,000 ppm males was significantly greater than that in the controls, but no other significant differences in sperm morphology or vaginal cytology were observed between exposed and control groups (Table I1).

The absolute and relative liver weights of 25,000 and 50,000 ppm males and the relative liver weights of 12,000 ppm males and of 25,000 and 50,000 ppm females were significantly greater than those of the controls (Table F1). No chemical-related gross or microscopic lesions were observed.

Dose Selection Rationale: Based on the absence of histopathologic effects after 13 weeks of exposure to up to 50,000 ppm phenolphthalein, the highest phenolphthalein exposure concentration selected for the 2-year feed study in rats was 50,000 ppm.

TABLE 3
Survival, Mean Body Weights, and Feed Consumption of Rats in the 13-Week Feed Study of Phenolphthalein

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	132 ± 4	351 ± 5	219 ± 6		16	18
3,000	10/10	128 ± 3	346 ± 6	218 ± 6	99	16	18
6,000	10/10	124 ± 2	349 ± 6	225 ± 5	99	15	17
12,000	10/10	129 ± 3	345 ± 7	216 ± 6	98	16	18
25,000	10/10	136 ± 4	344 ± 8	208 ± 6	98	16	19
50,000	10/10	129 ± 3	334 ± 5	205 ± 5	95	15	20
Female							
0	10/10	109 ± 3	210 ± 2	101 ± 2		13	11
3,000	10/10	105 ± 2	202 ± 5	97 ± 4	97	12	11
6,000	10/10	105 ± 2	208 ± 2	103 ± 2	99	13	12
12,000	9/9	107 ± 1	206 ± 3	100 ± 2	98	12	11
25,000	10/10	109 ± 3	198 ± 2	90 ± 2*	95	12	12
50,000	10/10	106 ± 3	198 ± 3*	92 ± 3*	95	12	12

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.

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 exposed males and females was similar to that of the controls.

Body Weights, Feed and Compound Consumption, and Clinical Findings

The mean body weights of exposed males were less than those of the controls through most of the second year of the study, and the mean body weights of exposed females were less than those of the controls from about week 16 until the end of the study (Figure 3, Tables 5 and 6). Feed consumption by all exposed groups of rats was similar to that by the controls (Tables K1 and K2). Dietary levels of 12,000, 25,000, or 50,000 ppm phenolphthalein resulted in average daily doses of approximately 500, 1,000, or 2,000 mg phenolphthalein/kg body weight to males and 500, 1,000, or 2,500 mg/kg to females. The estimated equivalent daily doses of phenolphthalein for the last year of the study ranged from 430 to 1,900 mg/kg for male rats and from 480 to 2,100 mg/kg for female rats. Clinical findings attributed to phenolphthalein exposure included thin appearance and ruffled fur in all exposed groups of males.

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

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	15	15	13	16
Natural deaths	14	20	22	21
Animals surviving to study termination	21	15	15[d]	13
Percent probability of survival at end of study[a]	42	30	30	26
Mean survival (days)[b]	640	652	658	662
Survival analysis[c]	P=0.463	P=0.492	P=0.626	P=0.405
Female				
Animals initially in study	50	50	50	50
Moribund	9	8	7	8
Natural deaths	11	4	11	4
Animals surviving to study termination	30	38	32	38
Percent probability of survival at end of study	60	76	64	76
Mean survival (days)	680	713	701	686
Survival analysis	P=0.278N	P=0.108N	P=0.679N	P=0.166N

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

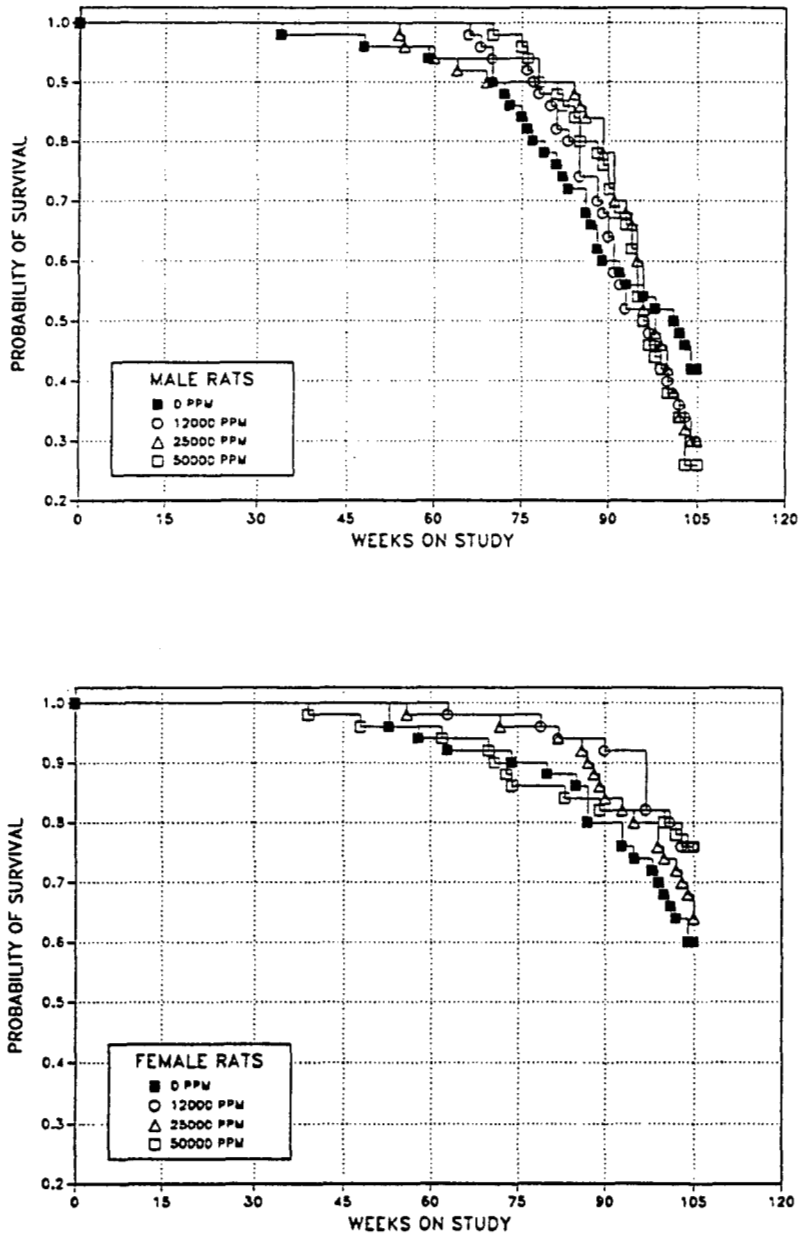


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered Phenolphthalein in Feed for 2 Years

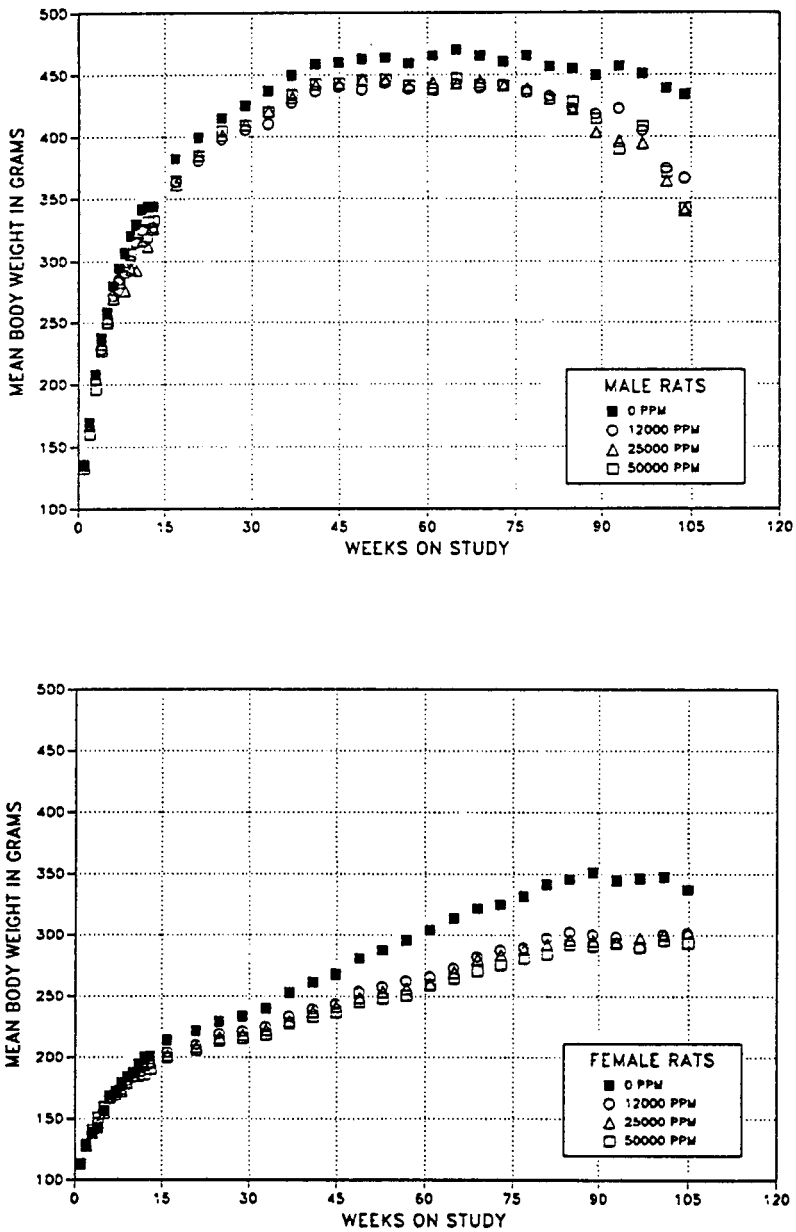


FIGURE 3
Growth Curves for Rats Administered Phenolphthalein in Feed for 2 Years

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

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	12,000 ppm Av. Wt. (g)	12,000 ppm Wt. (% of controls)	12,000 ppm No. of Survivors	25,000 ppm Av. Wt. (g)	25,000 ppm Wt. (% of controls)	25,000 ppm No. of Survivors	50,000 ppm Av. Wt. (g)	50,000 ppm Wt. (% of controls)	50,000 ppm No. of Survivors
1	135	50	135	100	50	136	100	50	133	98	50
2	170	50	168	99	50	168	99	50	161	95	50
3	208	50	203	98	50	204	98	50	197	94	50
4	237	50	229	96	50	233	98	50	227	96	50
5	258	50	250	97	50	254	98	50	249	97	50
6	280	50	272	97	50	270	96	50	269	96	50
7	294	50	286	97	50	286	97	50	283	96	50
8	306	50	289	94	50	276	90	50	292	95	50
9	320	50	301	94	50	294	92	50	306	95	50
10	330	50	315	96	50	293	89	50	316	96	50
11	341	50	325	95	50	317	93	50	326	95	50
12	344	50	318	93	50	312	91	50	332	97	50
13	344	50	327	95	50	326	95	50	333	97	50
17	382	50	364	95	50	362	95	50	365	95	50
21	400	50	381	95	50	386	97	50	385	96	50
25	415	50	398	96	50	402	97	50	405	98	50
29	425	50	406	95	50	410	97	50	409	96	50
33	437	50	411	94	50	421	96	50	420	96	50
37	449	49	428	95	50	432	96	50	434	97	50
41	459	49	437	95	50	442	96	50	443	97	50
45	460	49	440	96	50	442	96	50	443	96	50
49	463	48	438	95	50	446	97	50	446	96	50
53	464	48	443	96	50	445	96	50	447	96	50
57	459	48	439	96	50	441	96	48	442	96	50
61	466	47	439	94	50	444	95	47	439	94	50
65	470	47	443	94	50	443	94	46	447	95	50
69	466	47	440	95	48	445	96	46	442	95	50
73	461	44	442	96	47	441	96	45	442	96	49
77	465	41	436	94	46	439	94	45	437	94	47
81	457	38	433	95	41	431	94	45	431	94	45
85	455	36	423	93	40	422	93	44	428	94	42
89	450	31	419	93	35	404	90	42	415	92	39
93	457	29	423	93	28	397	87	35	390	86	34
97	451	27	405	90	25	395	88	26	409	91	24
101	439	26	375	85	20	364	83	21	372	85	19
104	434	21	367	85	15	341	79	15	342	79	13
Mean for weeks											
1-13	274		263	96		259	95		263	96	
14-52	432		411	95		416	96		417	97	
53-104	457		423	93		418	91		420	92	

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

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	12,000 ppm Av. Wt. (g)	12,000 ppm Wt. (% of controls)	12,000 ppm No. of Survivors	25,000 ppm Av. Wt. (g)	25,000 ppm Wt. (% of controls)	25,000 ppm No. of Survivors	50,000 ppm Av. Wt. (g)	50,000 ppm Wt. (% of controls)	50,000 ppm No. of Survivors
1	113	50	114	100	50	113	100	50	113	100	50
2	129	50	129	100	50	128	99	50	129	100	50
3	139	50	138	100	50	138	100	50	141	102	50
4	142	50	147	104	50	146	103	50	151	107	50
5	157	50	157	100	50	155	99	50	159	102	50
6	169	50	168	99	50	167	99	50	168	99	50
7	172	50	173	100	50	171	100	50	170	98	50
8	179	50	177	99	50	172	96	50	174	97	50
9	184	50	182	99	50	179	97	50	180	98	50
10	187	50	184	98	50	185	99	50	184	99	50
11	194	50	191	98	50	189	98	50	185	95	50
12	200	50	194	97	50	193	97	50	186	93	50
13	201	50	195	97	50	196	98	50	191	95	50
16	214	50	204	95	50	202	94	50	200	93	50
21	222	50	211	95	50	209	94	50	206	93	50
25	229	50	220	96	50	216	94	50	214	93	50
29	234	50	222	95	50	219	94	50	216	93	50
33	240	50	225	94	50	223	93	50	219	91	50
37	252	50	234	93	50	230	91	50	228	90	50
41	261	50	240	92	50	238	91	50	234	90	49
45	267	50	244	91	50	242	91	50	237	89	49
49	281	50	254	90	50	249	89	50	245	87	48
53	287	50	258	90	50	254	88	50	249	87	48
57	296	48	262	89	50	256	87	49	251	85	48
61	304	47	265	87	50	260	86	49	259	85	48
65	314	46	272	87	49	269	86	49	265	84	47
69	322	46	282	88	49	280	87	49	270	84	47
73	325	46	287	89	49	284	87	48	275	85	45
77	332	45	290	87	49	288	87	48	281	85	43
81	342	44	298	87	48	293	86	48	284	83	43
85	346	44	303	88	47	296	86	47	292	85	42
89	351	40	301	86	47	295	84	44	291	83	42
93	345	40	299	87	46	294	85	42	293	85	41
97	346	37	290	84	45	298	86	40	290	84	41
101	348	34	301	87	41	300	86	37	296	85	40
Mean for weeks											
1-13	167		165	99		164	98		164	98	
14-52	244		228	93		225	92		222	91	
53-101	328		285	87		282	86		277	84	

Determinations of Total Phenolphthalein in Plasma

On the last day of the 2-year study, blood was collected from the retroorbital sinus of three male and three female anesthetized rats from each group at five time points for the determination of plasma concentrations of total phenolphthalein (free and conjugated). The plasma concentrations of total phenolphthalein and their standard deviations in rats are given in Table H1. The semilogarithmic plots of plasma concentrations of total phenolphthalein (free and conjugated) versus time for male and female rats administered 12,000, 25,000, and 50,000 ppm for 2 years are shown and Figures H1 through H5. In general, the mean plasma concentrations of total phenolphthalein varied little with time of day. There were no significant differences in plasma concentrations of total phenolphthalein between exposure groups or between males and females.

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 adrenal medulla, kidney, seminal vesicle, spleen, mammary gland, pituitary gland, and other organs. 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.

Adrenal medulla: The incidences of benign pheochromocytoma of the adrenal medulla in all exposed groups of males were significantly greater than that in the controls and occurred with a significant positive trend (Tables 7 and A3). The incidences of

TABLE 7
Incidences of Neoplasms and Nonneoplastic Lesions of the Adrenal Medulla in Rats in the 2-Year Feed Study of Phenolphthalein

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Hyperplasia, Focal[a]	13	22*	18	23**
Benign Pheochromocytoma, Bilateral	3	19**	19**	15**
Benign Pheochromocytoma[b] (Includes Bilateral)				
Overall rate[c]	17/50 (34%)	34/50 (68%)	34/50 (68%)	34/50 (68%)
Adjusted rate[d]	62.1%	88.6%	93.7%	87.0%
Terminal rate[e]	11/21 (52%)	11/15 (73%)	13/15 (87%)	9/13 (69%)
First incidence (days)	608	485	372	528
Logistic regression test[f]	P=0.006	P<0.001	P<0.001	P=0.001
Malignant Pheochromocytoma (Includes Bilateral)	1	1	1	2
Benign or Malignant Pheochromocytoma				
Overall rate	18/50 (36%)	35/50 (70%)	35/50 (70%)	35/50 (70%)
Adjusted rate	62.9%	88.9%	93.9%	90.3%
Terminal rate	11/21 (52%)	11/15 (73%)	13/15 (87%)	10/13 (77%)
First incidence (days)	330	485	372	528
Logistic regression test	P=0.005	P<0.001	P<0.001	P=0.001

(continued)

TABLE 7
Incidences of Neoplasms and Nonneoplastic Lesions of the Adrenal Medulla in Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Female				
Number Examined Microscopically	50	50	50	49
Hyperplasia, Focal	10	18	15	11
Benign Pheochromocytoma, Bilateral	1	2	0	0
Benign Pheochromocytoma[g] (Includes Bilateral)				
Overall rate	3/50 (6%)	11/50 (22%)	9/50 (18%)	2/49 (4%)
Adjusted rate	10.0%	25.8%	23.7%	5.3%
Terminal rate	3/30 (10%)	7/38 (18%)	5/32 (16%)	2/38 (5%)
First incidence (days)	735 (T)	549	608	735 (T)
Logistic regression test	P=0.197N	P=0.031	P=0.074	P=0.392N
Malignant Pheochromocytoma	0	1	1	0
Benign or Malignant Pheochromocytoma[h]				
Overall rate	3/50 (6%)	12/50 (24%)	10/50 (20%)	2/49 (4%)
Adjusted rate	10.0%	28.2%	25.3%	5.3%
Terminal rate	3/30 (10%)	8/38 (21%)	5/32 (16%)	2/38 (5%)
First incidence (days)	735 (T)	549	568	735 (T)
Logistic regression test	P=0.189N	P=0.019	P=0.041	P=0.392N

(T)Terminal sacrifice
* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Historical incidence for 2-year NTP feed studies with untreated controls (mean ± standard deviation): 396/1,283 (30.9% ± 12.1%); range, 10%-63%
[c] Number of animals with neoplasm per number of animals with adrenal medulla examined microscopically
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[e] Observed incidence in animals surviving until the end of the study
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in an exposure group is indicated by N.
[g] Historical incidence: 50/1,274 (3.9% ± 2.4%); range, 0%-8%
[h] Historical incidence: 64/1,274 (5.0% ± 2.7%); range, 2%-12%

benign pheochromocytoma in 12,000 ppm females and of benign or malignant pheochromocytoma (combined) in 12,000 and 25,000 ppm females were significantly greater than those in the controls (Tables 7 and B3). The incidences of benign pheochromocytoma in all exposed groups of males and of benign pheochromocytoma and benign or malignant pheochromocytoma (combined) in 12,000 and 25,000 ppm females exceeded the ranges in historical controls in NTP 2-year feed studies (Tables 7, A4a, and B4a). The numbers of males with bilateral benign pheochromocytomas exceeded the number of controls with these neoplasms (Tables 7 and A1). The incidences of malignant pheochromocytomas in exposed rats were similar to those in the controls (Tables 7, A1, and B1). The incidences of focal hyperplasia of the adrenal medulla in 12,000 and 50,000 ppm males were significantly greater than that in the controls (Tables 7 and A5); however, the diagnosis of focal hyperplasia in an adrenal gland was made only in the absence of a diagnosis of a neoplasm in that adrenal gland. Therefore, exposed

groups were less at risk for diagnosis of hyperplasia. Focal hyperplasia, benign pheochromocytoma, and malignant pheochromocytoma are considered to be a morphologic continuum in the adrenal medulla. Focal hyperplasia consisted of irregular, small foci of small to normal-sized medullary cells arranged in packets or solid clusters slightly larger than normal; compression of surrounding parenchyma was minimal or absent. Benign pheochromocytomas were well-delineated masses often with altered architecture and variable compression of the surrounding parenchyma. Neoplastic cells were arranged in variably sized aggregates, clusters, and trabecular cords of varying thickness. Larger neoplasms usually exhibited greater cellular pleomorphism and atypia than smaller neoplasms. The few malignant pheochromocytomas were identified when there was invasion of, or beyond, the adrenal capsule.

Kidney: The incidences of proliferative lesions of the renal tubule epithelium in male rats were greater in all exposed groups than in the controls (Tables 8, A3, and A5). The incidences of renal tubule adenoma in 50,000 ppm males and of renal tubule adenoma or carcinoma (combined) in 12,000 and 50,000 ppm males were significantly greater than those in the controls (Tables 8 and A3). Although the neoplasms were predominantly adenomas, a few carcinomas were observed in exposed males (Tables 8 and A1). The incidences of renal tubule adenoma and adenoma or carcinoma (combined) in 12,000 and 50,000 ppm males exceeded the ranges in historical controls in NTP 2-year feed studies (Tables 7, A3, and A4b). Both renal tubule adenoma and carcinoma are relatively uncommon neoplasms in the male F344/N rat. Hyperplasia, adenoma, and carcinoma are thought to represent a continuum in the progression of proliferative lesions of the renal tubule epithelium. Hyperplasias were generally focal lesions characterized by increased numbers of renal tubule epithelial cells forming multiple layers that partially or totally filled the renal tubule lumen and usually caused dilation of the tubule. Generally, adenomas were discrete, expansile masses that were larger (greater than the diameter of five renal tubules) than the hyperplasias and had a more complex structure. Carcinomas were less discrete and larger than adenomas with hemorrhage, necrosis, and locally invasive growth patterns commonly observed.

One renal tubule adenoma was observed in a 50,000 ppm female at the standard evaluation (Tables 8 and B1). Because an effect was observed in males and renal tubule adenomas are rare in the female F344/N rat, an extended histologic evaluation (step sections) of the kidneys was conducted in females (Table 8). In this extended evaluation, no additional neoplasms were observed in the 25,000 or 50,000 ppm females, while neoplasms were identified in the 12,000 ppm group and the controls. Therefore, the adenoma in the 50,000 ppm female was not considered to be chemical related. For completeness, an extended evaluation was also conducted for male rats (Tables 8 and A3). In the expanded evaluation of males, additional adenomas were observed in exposed groups of males; only one additional adenoma was observed in the control males. This finding provides additional support that the increased incidences of renal tubule proliferative lesions in males were associated with the administration of phenolphthalein.

The incidences of nephropathy in all exposed groups of females were significantly greater than in the controls (Tables 8 and B5), and the severity of nephropathy in all exposed groups of males and in 25,000 and 50,000 ppm females was significantly greater than in the controls (Table 8). Nephropathy is a common spontaneously occurring lesion in aging F344/N rats, particularly males, and the typical morphological changes associated with nephropathy were present in this study. Changes consisted of the following spectrum of lesions: varying degrees of tubular dilation and distortion with cyst formation; proteinaceous tubular casts; atrophy; regeneration and hypertrophy of the renal tubule epithelium; thickening of the renal tubule and glomerular basement membranes; interstitial fibrosis; scattered foci of suppurative inflammation, primarily within degenerating renal tubules; and varying numbers and aggregates of mononuclear inflammatory cells within the interstitium. Marked renal tubule dilatation with cyst formation and transitional epithelial hyperplasia of the renal pelvis occur more commonly in kidneys with the most severe nephropathy, as was observed in exposed groups of males (Tables 8 and A5). The absence of a dose response for these two lesions is consistent with the similar severity of nephropathy observed in exposed groups.

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study of Phenolphthalein

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Single Sections (Standard Evaluation)				
Cyst[a]	1 (1.0)[b]	19** (1.8)	21** (1.7)	22** (1.9)
Nephropathy, Chronic	47 (1.8)	49 (2.9)**	50 (3.1)**	50 (3.1)**
Pelvis, Transitional Epithelium, Hyperplasia	4 (1.5)	31** (1.1)	34** (1.2)	29** (1.3)
Renal Tubule, Hyperplasia	0	6** (2.3)	7** (2.3)	2 (3.0)
Renal Tubule, Adenoma[c]				
Overall rate[d]	0/50 (0%)	4/50 (8%)	2/50 (4%)	6/50 (12%)
Adjusted rate[e]	0.0%	20.4%	10.6%	22.4%
Terminal rate[f]	0/21 (0%)	2/15 (13%)	1/15 (7%)	1/13 (8%)
First incidence (days)	—[h] 577	692 542		
Logistic regression test[g]	P=0.026	P=0.060	P=0.233	P=0.018
Renal Tubule, Carcinoma	0	1	1	2
Adenoma or Carcinoma[i]				
Overall rate	0/50 (0%)	5/50 (10%)	3/50 (6%)	7/50 (14%)
Adjusted rate	0.0%	24.0%	16.1%	24.7%
Terminal rate	0/21 (0%)	2/15 (13%)	1/15 (7%)	1/13 (8%)
First incidence (days)	— 577	692 542		
Logistic regression test	P=0.020	P=0.031	P=0.106	P=0.009
Step Sections (Extended Evaluation)				
Renal Tubule, Hyperplasia	3	23**	29**	27**
Renal Tubule, Adenoma				
Overall rate	1/50 (2%)	7/50 (14%)	15/50 (30%)	11/50 (22%)
Adjusted rate	4.8%	36.4%	64.9%	51.1%
Terminal rate	1/21 (5%)	4/15 (27%)	8/15 (53%)	5/13 (38%)
First incidence (days)	729 (T)	691 664	542	
Logistic regression test	P=0.003	P=0.017	P<0.001	P=0.003
Renal Tubule, Carcinoma	0	0	1	0
Renal Tubule, Adenoma or Carcinoma				
Overall rate	1/50 (2%)	7/50 (14%)	15/50 (30%)	11/50 (22%)
Adjusted rate	4.8%	36.4%	64.9%	51.1%
Terminal rate	1/21 (5%)	4/15 (27%)	8/15 (53%)	5/13 (38%)
First incidence (days)	729 (T)	691 664	542	
Logistic regression test	P=0.003	P=0.017	P<0.001	P=0.003
Single Sections and Step Sections (Combined)				
Renal Tubule, Hyperplasia	3	25**	29**	27**
Renal Tubule, Adenoma				
Overall rate	1/50 (2%)	10/50 (20%)	15/50 (30%)	15/50 (30%)
Adjusted rate	4.8%	46.9%	64.9%	61.8%
Terminal rate	1/21 (5%)	5/15 (33%)	8/15 (53%)	6/13 (46%)
First incidence (days)	729 (T)	577 664	542	
Logistic regression test	P<0.001	P=0.003	P<0.001	P<0.001
Renal Tubule, Carcinoma	0	1	2	2

(continued)

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male (continued)				
Single Sections and Step Sections (Combined) (continued)				
Renal Tubule, Adenoma or Carcinoma				
Overall rate	1/50 (2%)	10/50 (20%)	16/50 (32%)	16/50 (32%)
Adjusted rate	4.8%	46.9%	67.1%	62.9%
Terminal rate	1/21 (5%)	5/15 (33%)	8/15 (53%)	6/13 (46%)
First incidence (days)	729 (T)	577 664	542	
Logistic regression test	P<0.001	P=0.003	P<0.001	P<0.001
Female				
Number Examined Microscopically	50	50	50	50
Single Sections (Standard Evaluation)				
Nephropathy, Chronic	34 (1.2)	45* (1.4)	43* (1.5)*	44* (1.5)*
Renal Tubule, Adenoma[j]	0	0	0	1
Step Sections (Extended Evaluation)				
Renal Tubule, Hyperplasia	1	4	3	3
Renal Tubule, Adenoma	1	2	0	0
Single Sections and Step Sections (Combined)				
Renal Tubule, Hyperplasia	1	4	3	3
Renal Tubule, Adenoma	1	2	0	1

(T)Terminal sacrifice
* *Significantly different (P≤0.05) from the control group by the logistic regression test (incidence data) or the Mann-Whitney U test (severity of nephropathy)*
** P≤0.01
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Historical incidence for 2-year NTP feed studies with untreated controls (mean ± standard deviation): 9/1,301 (0.7% ± 1.5%); range, 0%-6%
[d] Number of animals with neoplasm per number of animals with kidney examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence: 12/1,301 (0.9% ± 1.5%); range, 0%-6%
[j] Historical incidence: 0/1,298 (0.0%)

Other organs: The incidences of diffuse hyperplasia of the parathyroid gland, fibrous osteodystrophy of the bone, and mineralization and degeneration of the glandular stomach in exposed groups of males were generally significantly greater than those in the controls (Tables 9 and A5). These lesions are commonly observed in male rats with more advanced nephropathy and are associated with a calcium/phosphorus imbalance created by compromised functional capacity of the kidney. The incidences of hyperplasia of the thyroid gland C-cells in 12,000 and 50,000 ppm males were significantly less than that in the controls and were also considered related to the calcium/phosphorus imbalance (Tables 9 and A5). Additionally, the incidence of mineralization of the aorta in 12,000 ppm males was significantly greater than that in the controls and was considered secondary to the renal disease.

TABLE 9
Incidences of Selected Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Phenolphthalein

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Blood Vessel[a]	50	49	50	50
Aorta, Media, Mineralization[b]	0	9**	3	4
Bone	50	50	50	50
Femur, Fibrous Osteodystrophy	0	17**	14**	12**
Parathyroid Gland	41	48	49	46
Hyperplasia, Diffuse	0	16**	14**	14**
Stomach, Glandular	50	50	50	49
Degeneration	0	11**	5*	4
Mineralization	0	11**	5*	5*
Thyroid Gland	50	50	49	49
C-cell, Hyperplasia, Focal	13	3**	9	4*

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion

Seminal vesicle: There were minimal increases in the incidences of atrophy of the seminal vesicle in exposed groups of males (0 ppm, 33/50; 12,000 ppm, 44/50; 25,000 ppm, 45/50; 50,000 ppm, 47/50; Table A5). The severity of the lesion was similar among exposed and control groups (2.5, 2.5, 2.8, 2.6). The atrophy consisted of reduced length of acinar branching, decreased size of secretory epithelial cells, increased fibrosis within the periacinar smooth muscle, and a decreased amount of eosinophilic secretory material. Sex glands are hormone dependent, and mild atrophy of the seminal vesicle associated with declining function of the testes is a common finding in aged male F344/N rats. Evaluation and interpretation of effects in primary sex organs and accessory sex glands in a 2-year study of male F344/N rats is complicated by the extremely high incidence of testicular interstitial cell neoplasms and associated degenerative changes. However, this effect would also be expected of an estrogenic exposure. The effect is consistent with a biological effect of phenolphthalein, but could also be explained by other changes in the animals. Chemical-related lesions were not observed in other accessory sex glands (e.g., prostate gland) in this study. The significance of this marginal increase and its association with the administration of phenolphthalein are uncertain.

Spleen: The incidences of lymphoid follicle atrophy of the spleen in exposed groups of males were greater than that in the controls (2/50, 8/50, 9/50, 12/49; Table A5). Macroscopically, affected spleens were observed to be smaller than normal, and histologically, there was a reduction in the mantle layer of lymphocytes. This change was observed predominantly in animals that died early or were killed moribund.

Mammary gland: The incidences of mammary gland fibroadenoma were decreased in exposed groups of females (0 ppm, 30/50; 12,000 ppm, 25/50; 25,000 ppm, 17/48; 50,000 ppm, 24/49; Table B3); however, the incidence in the control group (60%) slightly exceeded the range in historical controls in NTP 2-year feed studies [524/1,301 (40% ± 13%); range, 8%-58%]. There is a known association between the incidence of this neoplasm and body weight in female F344/N rats. The decreased incidences of this neoplasm were considered to be due primarily to the lower body weights of exposed groups and the unusually high incidence in the control group.

Pituitary gland: The incidence of adenoma of the pars distalis of the pituitary gland in 50,000 ppm females was significantly less than that in the controls (35/50, 32/49, 29/50, 25/47; Table B3), and the incidence of this lesion occurred with a significant negative trend. Pituitary gland adenoma in female F344/N rats occurs spontaneously at a high and variable rate in historical controls in NTP 2-year feed studies [666/1,290 (52% ± 13%); range, 30%-74%]. In this study, the incidence in the control group (70%) was at the upper end of the range for the historical controls, while the incidence in the 50,000 females (53%) was similar to the mean historical control incidence. Further, the dose-related decrease is inconsistent with the absence of a dose response in plasma phenolphthalein concentrations and other chemical-related effects in this study. Therefore, the marginal decrease of pituitary gland adenoma was not considered related to the administration of phenolphthalein.

MICE

14-DAY STUDY

All mice survived to the end of the study (Table 10). The final mean body weights of all exposed groups of mice were similar to those of the controls. Because of feed spillage, accurate measurements of feed and compound consumption could not be made. The feces of 100,000 ppm males and females were abnormally lighter in color than those of the other groups. This discoloration was observed on day 3 and continued throughout the study. No chemical-related gross or microscopic lesions were observed.

TABLE 10
Survival and Mean Body Weights of Mice in the 14-Day Feed Study of Phenolphthalein

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)		Final Weight Relative to Controls (%)
		Initial	Final	
Male				
0	5/5	24.4 ± 2.2	27.9 ± 3.2	
6,250	5/5	25.2 ± 1.8	28.2 ± 2.0	101
12,500	5/5	25.6 ± 1.8	29.6 ± 2.1	106
25,000	5/5	25.4 ± 1.9	28.9 ± 1.8	104
50,000	5/5	25.5 ± 1.7	28.5 ± 1.2	102
100,000	5/5	25.9 ± 2.3	26.5 ± 2.6	95
Female				
0	5/5	19.9 ± 0.9	23.0 ± 1.0	
6,250	5/5	20.1 ± 1.0	22.8 ± 1.1	99
12,500	5/5	19.9 ± 0.8	22.7 ± 0.8	99
25,000	5/5	20.3 ± 1.5	21.8 ± 0.6	95
50,000	5/5	19.9 ± 0.7	22.2 ± 0.8	97
100,000	5/5	19.9 ± 0.8	22.4 ± 0.8	97

[a] Number of animals surviving at 14 days/number initially in group. Differences from the control group are not significant by Williams' or Dunnett's test.
[b] Weights are given as mean ± standard deviation.

13-WEEK STUDY

All mice survived until the end of the study (Table 11). The final mean body weights and body weight gains of all exposed groups were similar to those of the controls. Feed consumption by exposed groups of mice was similar to that by the controls. Dietary levels of 3,000, 6,000, 12,000, 25,000, or 50,000 ppm phenolphthalein resulted in average daily doses of approximately 500, 1,000, 2,000, 4,100, or 9,000 mg phenolphthalein/kg body weight to males and 600, 1,200, 2,400, 5,000, or 10,500 mg/kg to females. There was no cathartic action nor any other clinical finding attributed to exposure to phenolphthalein.

TABLE 11
Survival, Mean Body Weights, and Feed Consumption of Mice in the 13-Week Feed Study of Phenolphthalein

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	20.4 ± 0.7	27.8 ± 0.8	7.4 ± 0.5		3.7	3.9
3,000	10/10	19.8 ± 0.5	25.8 ± 0.6	6.1 ± 0.5	93	4.0	4.3
6,000	10/10	20.3 ± 0.5	26.8 ± 0.6	6.6 ± 0.3	97	3.8	4.4
12,000	10/10	19.9 ± 0.5	27.4 ± 0.5	7.5 ± 0.4	99	3.9	4.1
25,000	10/10	19.6 ± 0.5	27.6 ± 0.7	7.9 ± 0.4	99	4.3	4.4
50,000	10/10	20.0 ± 0.4	26.5 ± 0.6	6.5 ± 0.4	95	4.3	4.6
Female							
0	10/10	15.8 ± 0.7	22.9 ± 0.6	7.1 ± 0.3		3.5	4.5
3,000	10/10	16.6 ± 0.5	23.9 ± 0.7	7.3 ± 0.4	104	4.4	4.5
6,000	10/10	17.7 ± 0.4*	24.1 ± 0.5	6.4 ± 0.3	105	4.0	4.6
12,000	10/10	16.9 ± 0.4	23.8 ± 0.4	6.9 ± 0.3	104	4.0	4.6
25,000	10/10	17.1 ± 0.3	24.0 ± 0.4	6.9 ± 0.3	105	4.1	4.8
50,000	10/10	17.0 ± 0.4	23.6 ± 0.5	6.7 ± 0.3	103	4.1	5.0

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.

The absolute right cauda weight of the 12,000 ppm males was significantly less than that of the controls (Table I2). The absolute right epididymis weights of 12,000, 25,000, and 50,000 ppm males were significantly less than that of the controls. The percentages of abnormal sperm in 12,000, 25,000, and 50,000 ppm males were significantly greater than that in the control group, and the sperm concentrations in 12,000 and 50,000 ppm males were significantly less than that in the control group. The estrous cyclicity appeared normal in females (Table I2). In some groups of females, there was a slight lengthening of the cycle, but a specific phase could not be identified as being responsible.

The absolute and relative right testis weights of males exposed to 6,000 ppm or greater and the absolute right testis weight of 3,000 ppm males were significantly less than those of the control group (Table F2). Other organ weight differences in mice were attributed to changes in body weights of exposed mice and were not considered to be related to exposure to phenolphthalein. Although a very subtle lesion in most instances, some seminiferous tubules lacked one or more generations of germ cells in portions or the entirety of a seminiferous tubule. This could be seen by the presence of spermatogonia, early spermatocytes, and pachytene spermatocytes, but no round or elongated spermatids. Similarly, other seminiferous tubules contained Sertoli nuclei, spermatogonia, early spermatocytes, and elongating spermatids, but no pachytene spermatocytes or round spermatids. Occasionally, seminiferous tubules contained only Sertoli cells. Often an affected seminiferous tubule would be surrounded by tubules that looked normal.

The incidences of hypoplasia of the bone marrow in males and females exposed to 12,000 ppm or greater were significantly greater than those in the controls (Table 12). This lesion was characterized by less cellular marrow with a decreased myeloid to erythroid ratio, vascular congestion, and individual cell necrosis. The identification of the necrotic cell type was not always possible, but in many instances, these cells appeared to be myeloid precursors. In addition, there was a slight increase in pigment interpreted as hemosiderin. The incidences of hematopoiesis of the spleen in 25,000 and 50,000 ppm males were significantly greater than that in the controls (Table 12). The bone marrow and spleen findings were minimal to mild in severity.

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Mice in the 13-Week Feed Study of Phenolphthalein

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male						
Bone Marrow[a]	10	10	10	10	10	10
Hypoplasia[b]	0	0	1 (1.0)[c]	10** (1.0)	10** (1.0)	10** (2.0)
Necrosis	0	0	1 (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Spleen	10	—[d]	—	10	10	10
Hematopoiesis	0	—	—	0	9** (1.0)	10** (1.0)
Female						
Bone Marrow	10	—	10	10	10	10
Hypoplasia	0	—	0	8** (1.0)	10** (1.0)	10** (1.0)
Necrosis	0	—	0	8** (1.0)	10** (1.0)	10** (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 of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[d] Tissue not examined at this exposure concentration

Dose Selection Rationale: Based on the minimal severity of bone marrow and splenic lesions after 13 weeks of exposure to 12,000 ppm, the highest phenolphthalein exposure concentration selected for the 2-year feed study in mice was 12,000 ppm.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 13 and in the Kaplan-Meier survival curves (Figure 4). Survival of the 12,000 ppm females was significantly lower than that of the controls; survival of all other exposed groups of mice was similar to that of the controls.

Body Weights, Feed and Compound Consumption, and Clinical Findings

The mean body weights of 12,000 ppm males were slightly less than those of the controls beginning at week 93 of the study, and the mean body weights of the 3,000, 6,000, and 12,000 ppm females were less than those of the controls during most of the second year of the study (Figure 5, Tables 14 and 15). Feed consumption by all exposed groups of mice was similar to that by the controls (Tables K3 and K4). Dietary levels of 3,000, 6,000, or 12,000 ppm phenolphthalein resulted in average daily doses of approximately 300, 600, or 1,200 mg phenolphthalein/kg body weight to males and 400, 800, or 1,500 mg/kg to females. The estimated equivalent daily doses of phenolphthalein for the last year of the study ranged from 290 to 1,200 mg/kg for male mice and from 310 to 1,300 mg/kg for female mice. In exposed mice, there were no clinical findings related to phenolphthalein exposure.

Determinations of Total Phenolphthalein in Plasma

On the last day of the 2-year study, blood was collected from the retroorbital sinus of three male and three female anesthetized mice from each group at five time points for the determination of plasma concentrations of total phenolphthalein (free and conjugated). The plasma concentrations of total phenolphthalein and their standard deviations in mice are given in Table H2. The semilogarithmic plots of plasma concentration of total phenolphthalein (free and conjugated) versus time data for male and female mice administered 3,000, 6,000, and 12,000 ppm for 2 years are shown in Table H2 and Figures H6 through H10. In general, the mean plasma concentrations of total phenolphthalein varied little with time of day. There were no significant differences in plasma concentrations of total phenolphthalein between exposure groups or between males and females.

TABLE 13
Survival of Mice in the 2-Year Feed Study of Phenolphthalein

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male				
Animals initially in study	50	50	50	50
Missing[a]	0	0	0	1
Moribund	6	13	11	3
Natural deaths	4	4	3	10
Animals surviving to study termination	40	33	36	36
Percent probability of survival at end of study[b]	80	66	72	74
Mean survival (days)[c]	701	695	695	681
Survival analysis[d]	P=0.826	P=0.186	P=0.461	P=0.600
Female				
Animals initially in study	50	50	50	50
Moribund	5	13	9	12
Natural deaths	6	6	7	10
Animals surviving to study termination	39[e]	31	34	28
Percent probability of survival at end of study	78	62	68	56
Mean survival (days)	697	665	687	663
Survival analysis	P=0.047	P=0.097	P=0.331	P=0.024

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

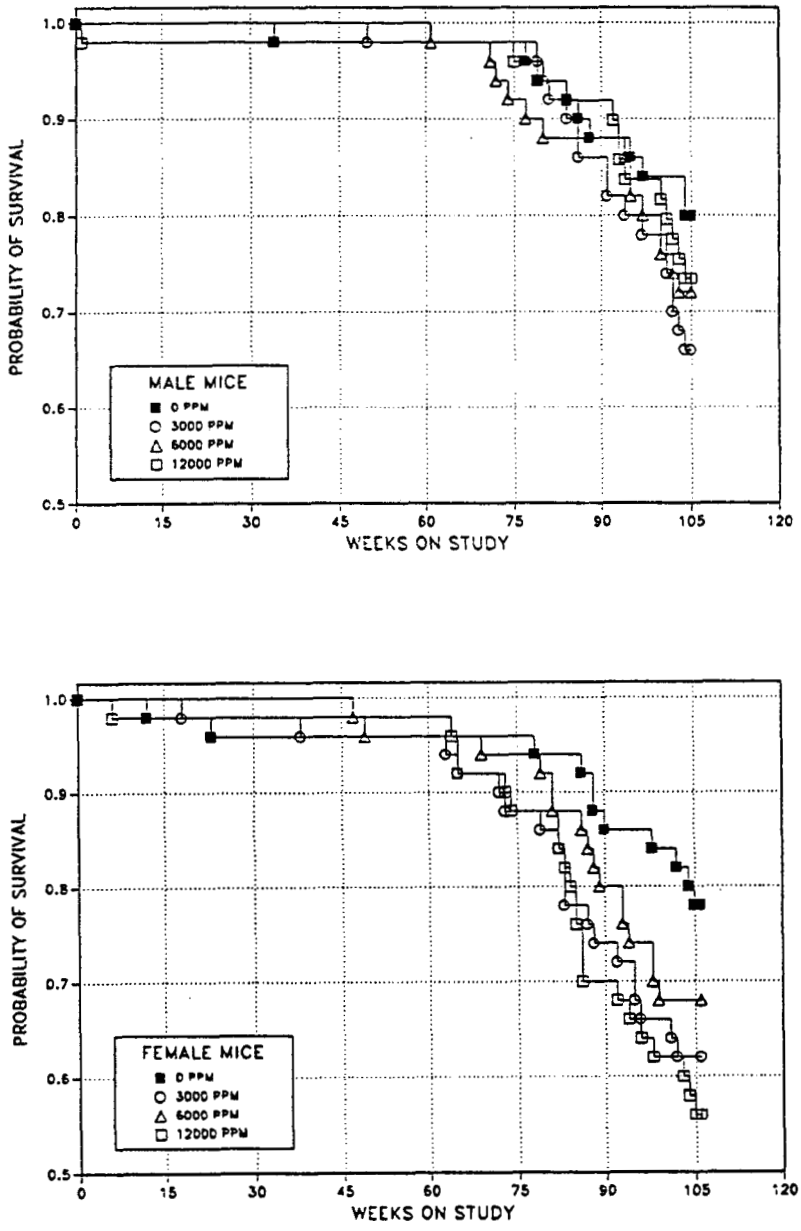


FIGURE 4
Kaplan-Meier Survival Curves for Mice Administered Phenolphthalein in Feed for 2 Years

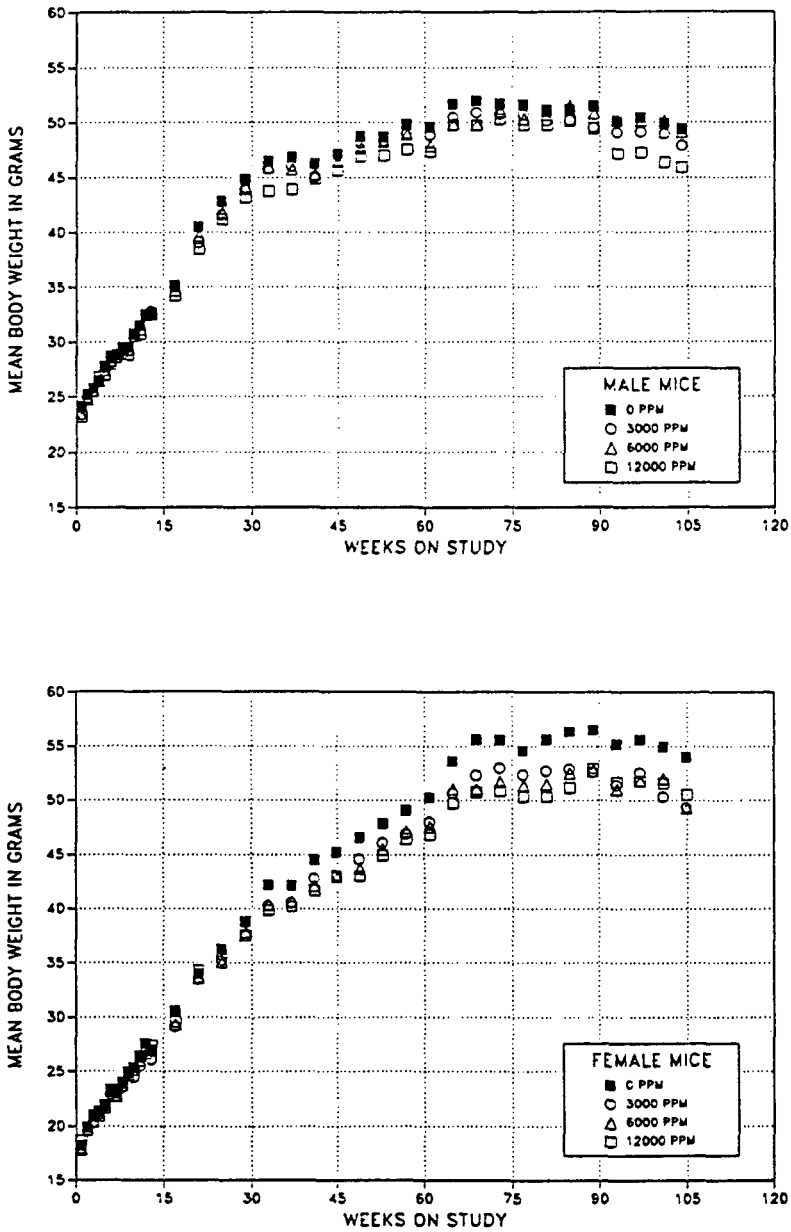


FIGURE 5
Growth Curves for Mice Administered Phenolphthalein in Feed for 2 Years

TABLE 14
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of Phenolphthalein

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	3,000 ppm Av. Wt. (g)	3,000 ppm Wt. (% of controls)	3,000 ppm No. of Survivors	6,000 ppm Av. Wt. (g)	6,000 ppm Wt. (% of controls)	6,000 ppm No. of Survivors	12,000 ppm Av. Wt. (g)	12,000 ppm Wt. (% of controls)	12,000 ppm No. of Survivors
1	24.1	50	23.4	97	50	23.2	96	50	23.2	96	50
2	25.2	50	24.9	99	50	24.8	98	50	24.8	98	49
3	25.8	50	25.5	99	50	25.5	99	50	25.5	99	49
4	26.4	50	26.4	100	50	26.4	100	50	26.8	102	49
5	27.7	50	27.4	99	50	27.1	98	50	26.9	97	48
6	28.7	50	28.1	98	50	28.3	99	50	28.0	98	48
7	28.9	50	28.5	99	50	28.6	99	50	28.8	100	48
8	29.5	50	28.8	98	50	29.5	100	50	29.2	99	48
9	29.5	50	29.2	99	50	29.4	100	50	28.8	98	48
10	30.7	50	30.4	99	50	30.6	100	50	30.8	100	48
11	31.5	50	31.5	100	50	31.1	99	50	30.7	98	48
12	32.5	50	32.4	100	50	32.5	100	50	32.4	100	48
13	32.6	50	32.8	101	50	32.6	100	50	32.5	100	48
17	35.2	50	35.0	99	50	34.7	99	50	34.2	97	48
21	40.5	50	39.1	97	50	39.7	98	50	38.5	95	48
25	42.8	50	41.6	97	50	41.8	98	50	41.2	96	48
29	44.8	50	43.9	98	50	44.0	98	50	43.2	96	48
33	46.5	50	45.7	98	50	45.9	99	50	43.8	94	48
37	46.8	49	46.0	98	50	45.7	98	50	43.9	94	48
41	46.2	49	45.0	97	50	45.3	98	50	44.9	97	48
45	47.1	49	47.0	100	50	46.4	99	50	45.6	97	48
49	48.7	49	48.2	99	50	47.7	98	50	46.9	96	48
53	48.7	49	48.3	99	49	48.3	99	50	47.0	97	48
57	49.8	49	49.0	98	49	48.9	98	50	47.5	95	48
61	49.6	49	48.9	99	49	47.9	97	50	47.3	95	48
65	51.7	49	50.5	98	49	49.8	96	49	49.8	96	48
69	52.0	49	50.9	98	49	49.8	96	49	49.8	96	48
73	51.7	49	50.7	98	49	51.0	99	47	50.3	97	48
77	51.6	48	50.7	98	49	50.3	98	46	49.8	97	47
81	51.1	47	50.2	98	47	51.0	100	44	49.8	98	46
85	51.1	46	50.2	98	45	51.5	101	44	50.1	98	45
89	51.5	45	49.5	96	43	50.8	99	44	49.4	96	45
93	50.0	44	49.1	98	41	50.2	100	44	47.1	94	44
97	50.4	43	49.1	97	40	50.1	99	41	47.3	94	41
101	49.9	42	49.0	98	39	50.2	101	38	46.3	93	40
104	49.4	40	47.9	97	33	49.1	99	36	45.9	93	36
Mean for weeks											
1-13	28.7		28.4	99		28.4	99		28.3	99	
14-52	44.3		43.5	98		43.5	98		42.5	96	
53-104	50.6		49.6	98		49.9	99		48.4	96	

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

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	3,000 ppm Av. Wt. (g)	3,000 ppm Wt. (% of controls)	3,000 ppm No. of Survivors	6,000 ppm Av. Wt. (g)	6,000 ppm Wt. (% of controls)	6,000 ppm No. of Survivors	12,000 ppm Av. Wt. (g)	12,000 ppm Wt. (% of controls)	12,000 ppm No. of Survivors
1	18.2	50	17.9	98	50	17.8	98	50	18.7	103	50
2	19.9	50	19.5	98	50	19.6	99	50	19.6	99	50
3	20.9	50	20.3	97	50	20.4	98	50	21.0	101	50
4	21.3	50	21.1	99	50	21.0	99	50	21.1	99	50
5	21.8	50	21.8	100	50	21.7	100	50	21.9	101	50
6	23.4	50	23.0	98	50	23.2	99	50	23.4	100	50
7	23.0	50	22.7	99	50	22.7	99	50	23.3	101	49
8	24.0	50	23.5	98	50	23.8	99	50	24.0	100	49
9	24.9	50	24.3	98	50	24.7	99	50	24.9	100	49
10	25.4	50	24.5	97	50	25.2	99	50	25.1	99	49
11	26.5	50	25.5	96	50	26.0	98	50	26.4	100	49
12	27.6	49	26.9	98	50	26.7	97	50	26.9	98	49
13	27.0	49	26.1	97	50	26.9	100	50	27.4	102	49
17	30.6	49	29.1	95	50	29.3	96	50	29.6	97	49
21	34.0	49	33.6	99	49	33.6	99	50	34.3	101	49
25	36.2	48	35.2	97	49	35.1	97	50	35.8	99	49
29	38.8	48	37.5	97	49	37.8	97	50	37.5	97	49
33	42.2	48	40.3	96	49	40.3	96	50	39.9	95	49
37	42.1	48	40.6	96	49	40.7	97	50	40.2	96	49
41	44.5	48	42.8	96	48	42.1	95	50	41.7	94	49
45	45.2	48	43.1	95	48	42.9	95	50	43.1	95	49
49	46.6	48	44.6	96	48	43.7	94	49	43.0	92	49
53	47.8	48	46.1	96	48	45.5	95	48	45.0	94	49
57	49.0	48	46.9	96	48	47.1	96	48	46.5	95	49
61	50.2	48	48.0	96	48	47.6	95	48	46.8	93	49
65	53.6	48	50.7	95	47	51.1	95	48	49.7	93	48
69	55.6	48	52.3	94	46	51.0	92	48	50.8	91	46
73	55.6	48	52.9	95	45	51.7	93	47	50.9	92	46
77	54.6	48	52.4	96	44	51.4	94	47	50.3	92	44
81	55.6	47	52.7	95	43	51.5	93	46	50.3	91	44
85	56.3	47	52.9	94	39	52.5	93	44	51.2	91	39
89	56.5	44	52.6	93	37	52.7	93	41	52.9	94	35
93	55.2	43	51.4	93	36	51.0	92	39	51.7	94	34
97	55.6	43	52.5	94	33	51.8	93	37	51.8	93	32
101	54.9	42	50.3	92	33	52.0	95	34	51.6	94	31
Mean for weeks											
1-13	23.4		22.9	98		23.1	99		23.4	100	
14-52	40.0		38.5	96		38.4	96		38.3	96	
53-101	53.9		50.9	94		50.5	94		50.0	93	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of histiocytic sarcoma and malignant lymphoma and neoplasms and/or nonneoplastic lesions of the thymus, ovary, testis, bone marrow, kidney, spleen, liver, tooth, pituitary gland, and thyroid gland. 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.

All organs: The incidences of histiocytic sarcoma in 6,000 and 12,000 ppm males and females were significantly greater than those in the controls and occurred with a significant positive trend (Tables 16, C3, and D3). The incidences of histiocytic sarcoma in all exposed groups of males and in 6,000 and

TABLE 16
Incidences of Histiocytic Sarcoma in Mice in the 2-Year Feed Study of Phenolphthalein

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male[a]				
Overall rate[b]	1/50 (2%)	3/50 (6%)	11/50 (22%)	12/49 (24%)
Adjusted rate[c]	2.4%	7.9%	28.0%	27.5%
Terminal rate[d]	0/40 (0%)	0/33 (0%)	8/36 (22%)	5/36 (14%)
First incidence (days)	727	658	665	549
Life table test[e]	P<0.001	P=0.255	P=0.002	P=0.002
Logistic regression test[e]	P<0.001	P=0.302	P=0.002	P=0.001
Female[f]				
Overall rate	0/50 (0%)	2/50 (4%)	7/50 (14%)	7/50 (14%)
Adjusted rate	0.0%	6.5%	17.6%	18.0%
Terminal rate	0/39 (0%)	2/31 (6%)	3/34 (9%)	0/28 (0%)
First incidence (days)	—[g]	737 (T)	567	588
Life table test	P=0.002	P=0.189	P=0.007	P=0.005
Logistic regression test	P=0.009	P=0.189	P=0.010	P=0.011

(T)Terminal sacrifice

[a] Historical incidence for 2-year NTP feed studies with untreated controls (mean ± standard deviation): 6/1,474 (0.4% ± 1.0%); range, 0%-2%

[b] Number of animals with neoplasm per number of animals necropsied

[c] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

[d] Observed incidence in animals surviving until the end of the study

[e] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal.

[f] Historical incidence: 19/1,473 (1.3% ± 1.6%); range, 0%-4%

[g] Not applicable; no neoplasms in animal group

12,000 ppm females exceeded the ranges in historical controls in NTP 2-year feed studies (Tables 16, C4a, and D4a). In this study, histiocytic sarcoma was consistently observed in the liver with several other sites (e.g., spleen, lung, bone marrow, and various lymph nodes) involved less frequently. In the liver, hepatic sinusoids were expanded by neoplastic histiocytes, which frequently formed small nodules, and occasional thick bands, which often effaced hepatic parenchyma (Plates 1 and 2). Nuclei of neoplastic cells varied from rounded to kidney-shaped, and cells contained abundant pale eosinophilic cytoplasm.

The incidences of all types of malignant lymphoma in all exposed groups of females were significantly greater than that in the controls and exceeded the range in historical controls in NTP 2-year feed studies (Tables 17, D3, and D4b). The incidences in all exposed groups of males were similar to that in the controls (Tables 17 and C3).

In this study, many proliferative lesions (atypical hyperplasia and lymphoma) that clearly arose within the thymus were observed in exposed males and females. Lymphomas were considered to be of thymic origin when they were observed only in the

TABLE 17
Incidences of Malignant Lymphoma and of Selected Thymic Neoplasms and Nonneoplastic Lesions in Mice in the 2-Year Feed Study of Phenolphthalein

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male				
Malignant Lymphoma, All Types[a]				
Overall rate[b]	6/50 (12%)	8/50 (16%)	12/50 (24%)	8/49 (16%)
Adjusted rate[c]	14.5%	19.1%	27.2%	22.2%
Terminal rate[d]	5/40 (13%)	3/33 (9%)	6/36 (17%)	8/36 (22%)
First incidence (days)	662	347	423	729 (T)
Life table test[e]	P=0.299	P=0.300	P=0.082	P=0.309
Logistic regression test[e]	P=0.324	P=0.396	P=0.101	P=0.353
Thymus[f]	43	46	44	42
Atypical Hyperplasia[g]	0	3	7**	7**
Lymphoma, Primary Site Thymus				
Overall rate	0/50 (0%)	2/50 (4%)	7/50 (14%)	1/49 (2%)
Adjusted rate	0.0%	4.9%	15.4%	2.8%
Terminal rate	0/40 (0%)	0/33 (0%)	2/36 (6%)	1/36 (3%)
First incidence (days)	—[h]	583	423	729 (T)
Life table test	P=0.357	P=0.223	P=0.010	P=0.479
Logistic regression test	P=0.412	P=0.234	P=0.006	P=0.479
Lymphoma, Primary Site Thymus or Probably Thymus				
Overall rate	0/50 (0%)	4/50 (8%)	7/50 (14%)	2/49 (4%)
Adjusted rate	0.0%	9.8%	15.4%	5.6%
Terminal rate	0/40 (0%)	1/33 (3%)	2/36 (6%)	2/36 (6%)
First incidence (days)	—	583	423	729 (T)
Life table test	P=0.317	P=0.055	P=0.010	P=0.215
Logistic regression test	P=0.359	P=0.061	P=0.006	P=0.215

(continued)

TABLE 17
Incidences of Malignant Lymphoma and of Selected Thymic Neoplasms and Nonneoplastic Lesions in Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Female				
Malignant Lymphoma, All Types[i]				
Overall rate	15/50 (30%)	28/50 (56%)	33/50 (66%)	25/50 (50%)
Adjusted rate	34.3%	60.2%	74.6%	65.8%
Terminal rate	11/39 (28%)	13/31 (42%)	23/34 (68%)	16/28 (57%)
First incidence (days)	156	266	338	447
Life table test	P=0.010	P=0.003	P<0.001	P=0.004
Logistic regression test	P=0.114	P=0.051	P<0.001	P=0.035
Thymus	48	44	49	45
Atypical Hyperplasia	0	7**	6**	5**
Lymphoma, Primary Site Thymus				
Overall rate	0/50 (0%)	5/50 (10%)	5/50 (10%)	4/50 (8%)
Adjusted rate	0.0%	13.9%	13.8%	12.6%
Terminal rate	0/39 (0%)	3/31 (10%)	4/34 (12%)	3/28 (11%)
First incidence (days)	—	266	604	450
Life table test	P=0.086	P=0.023	P=0.025	P=0.037
Logistic regression test	P=0.157	P=0.038	P=0.030	P=0.065
Lymphoma, Primary Site Thymus or Probably Thymus				
Overall rate	1/50 (2%)	9/50 (18%)	10/50 (20%)	7/50 (14%)
Adjusted rate	2.6%	24.9%	25.8%	23.1%
Terminal rate	1/39 (3%)	6/31 (19%)	7/34 (21%)	6/28 (21%)
First incidence (days)	737 (T)	266	551	450
Life table test	P=0.044	P=0.005	P=0.004	P=0.011
Logistic regression test	P=0.106	P=0.011	P=0.005	P=0.025

(T)Terminal sacrifice
** Significantly different ($P \leq 0.01$) from the control group by the logistic regression test
[a] Historical incidence for 2-year NTP feed studies with untreated controls (mean ± standard deviation): 130/1,474 (8.8% ± 6.0%); range, 2%-24%
[b] Number of animals with neoplasm per number of animals necropsied
[c] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[d] Observed incidence in animals surviving until the end of the study
[e] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal.
[f] Number of animals with thymus examined microscopically
[g] Number of animals with lesion
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence: 339/1,473 (23.0% ± 11.9%); range, 6%-44%

thymus or within the thymus and metastatic only to other sites within the chest cavity. The incidences of lymphoma of thymic origin were increased in exposed groups of males, but were significantly increased only in the 6,000 ppm group, and the incidences of lymphoma of thymic origin in all exposed groups of females were significantly greater (by the life table test) than that in the controls (Table 17). In the other exposed groups, where the lymphoma was largest in the thymus with minimal involvement of other sites, the thymus was considered the "probable" site of origin. Additionally, the incidences of atypical hyperplasia of the thymus in 6,000 and 12,000 ppm males and in all exposed groups of females were significantly greater than those in the controls.

After puberty, there is a gradual involution of the bilobed thymic tissue with advancing age, and while the amount of thymic tissue is reduced at 2 years, the morphological structure of the outer cortex and inner medulla is generally maintained. The thymic cortex is generally composed of smaller hyperchromatic lymphocytes, while more eosinophilic staining lymphocytes and epithelial cells are major constituents of the medulla. In this study, animals with hyperplasia of the thymus often had a normal appearing lobe along with an abnormal lobe (Plates 3 and 4). The abnormal lobe was generally smaller than the normal lobe and lacked a distinct cortex and medulla. Rather, abnormal lobes were composed of sheets of large lymphocytes combined with variable numbers of smaller, more normal appearing lymphocytes (Plate 5). The large lymphocytes had stippled chromatin, scant to moderate amounts of cytoplasm, and one or two fairly distinct nucleoli. The mitotic index was variable. In the earliest lesions diagnosed as malignant lymphomas, a fairly homogeneous population of lymphocytes (as described previously) extended beyond the confines of normal thymic tissue, and mitotic figures were common. Larger thymic lymphomas occupied a larger portion of the chest cavity.

Ovary: There were increased incidences of benign ovarian neoplasms and associated hyperplasia of a rather distinctive morphology in all exposed groups of females (Tables 18, D1, D3, and D5). The incidences of benign sex-cord stromal tumors of the ovary in all exposed groups of females were significantly greater than that in the controls and exceeded the range for ovarian luteomas in historical controls in NTP 2-year feed studies (Tables 18, D3, and D4c). The incidences of hyperplasia of the ovary in 3,000 and 12,000 ppm females were significantly greater than that in the controls. The neoplasms varied from occupying most of the ovary to markedly expanding it (three to four times) with compression of the surrounding parenchyma (Plates 6 and 7). Neoplasms were usually composed of sheets of round to oval cells with abundant cytoplasm that varied from finely granular and eosinophilic to vesiculated (luteinized). In most neoplasms, the cells were rather homogeneous with a low mitotic index; however, in some of the larger neoplasms, there was a modest degree of cellular pleomorphism and a higher mitotic rate. Hyperplasias were observed within the ovarian stroma and were composed of cells that were morphologically similar to those in the neoplasms (Plates 8 and 9). However, hyperplasias were smaller, and component cells usually blended with the surrounding parenchyma with little or no compression. The histomorphology of the granulosa cell tumors identified in a control female and in a 3,000 ppm female was typical of granulosa cell tumors and was distinctly different from the benign sex-cord stromal tumors observed in exposed females.

TABLE 18
Incidences of Neoplasms and Nonneoplastic Lesions of the Ovary in Female Mice in the 2-Year Feed Study of Phenolphthalein

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Number Examined Microscopically	50	49	50	50
Hyperplasia[a]	4 (1.5)[b]	11* (2.0)	10 (2.6)	17** (2.2)
Benign Sex-Cord Stromal Tumor, Bilateral	0	1	0	0
Benign Sex-Cord Stromal Tumor[c] (Includes Bilateral)				
Overall rate[d]	0/50 (0%)	7/49 (14%)	6/50 (12%)	5/50 (10%)
Adjusted rate[e]	0.0%	21.7%	17.6%	17.9%
Terminal rate[f]	0/39 (0%)	6/31 (19%)	6/34 (18%)	5/28 (18%)
First incidence (days)	—[h]	668	737 (T)	737 (T)
Logistic regression test[g]	P=0.066	P=0.004	P=0.011	P=0.012
Benign Granulosa Cell Tumor	1	1	0	0

(T)Terminal sacrifice
* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Historical incidence of ovarian luteoma for 2-year NTP feed studies with untreated controls (mean ± standard deviation): 5/1,436 (0.4% ± 1.0%); range, 0%-4%
[d] Number of animals with neoplasm per number of animals with ovary examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal.
[h] Not applicable; no neoplasms in animal group

Testis: The incidences of germinal epithelial degeneration of the testis in all exposed groups of males were significantly greater than in the controls (Tables 19 and C5). The lesion varied from involvement of a few to all seminiferous tubules. Within the tubules, there was variable loss of sperm and germinal epithelial cells (spermatogonia, spermatocytes, and spermatids), often with only Sertoli cells remaining (Plates 10 and 11). In the most severe cases, the cross-sectional diameter of the testis was decreased. Although more severe, this lesion is consistent with that observed in the 13-week study.

Bone marrow: There were increased incidences of myelofibrosis of the bone marrow in 12,000 ppm males (Tables 19 and C5) and an increased severity of this lesion in exposed females (Table 19). In this study, the fibro-osseous lesion generally partially replaced the bone marrow cavity and was composed of minimal to mild proliferation of spindle cells in an eosinophilic, often fibrillar, matrix. In advanced lesions, the spindle cell proliferation was more extensive, and there was more osteosclerosis. Additionally, as observed in the 13-week study, there were increased incidences of pigmentation (probably

TABLE 19
Incidences of Selected Nonneoplastic Lesions in Mice in the 2-Year Feed Study of Phenolphthalein

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male				
Bone Marrow[a]	50	50	50	49
Myelofibrosis[b]	3 (1.0)[c]	8 (1.3)	8 (1.3)	19** (1.3)
Pigmentation	0	2 (1.0)	5* (1.8)	16** (1.8)
Kidney	50	50	50	49
Renal Tubule, Accumulation, Hyaline Droplet	0	2 (2.5)	5* (2.6)	5* (2.8)
Spleen	50	50	50	49
Hematopoietic Cell Proliferation	10 (1.9)	22* (2.3)	28** (2.5)	21* (2.6)
Testes	50	50	50	48
Germinal Epithelium, Degeneration	1 (1.0)	49** (2.0)	50** (3.0)	47** (2.7)
Female				
Bone Marrow	50	50	50	50
Myelofibrosis	34 (1.4)	34 (2.7)	38 (2.0)	36 (2.3)
Pigmentation	2 (1.5)	3 (1.3)	11** (1.9)	11** (2.2)
Kidney	50	50	50	50
Renal Tubule, Accumulation, Hyaline Droplet	0	1 (1.0)	3 (2.0)	8** (2.0)
Spleen	50	50	50	50
Hematopoietic Cell Proliferation	13 (2.8)	14 (2.5)	20 (2.7)	21 (2.5)

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

hemosiderin) of minimal to mild severity in the bone marrow of 6,000 and 12,000 ppm males and females (Tables 19, C5, and D5).

Kidney: The incidences of hyaline droplets within the renal tubules in 6,000 and 12,000 ppm males and in 12,000 ppm females were significantly greater than those in the controls (Tables 19, C5, and D5). Mild to moderate numbers of eosinophilic, intracytoplasmic hyaline droplets were observed within the proximal convoluted renal tubules. Generally, mice with renal hyaline droplets also had histiocytic sarcoma in other organs. This renal change is not uncommon in mice with histiocytic sarcomas. It has been shown that histiocytic sarcomas may produce excessive amounts of lysozyme, which may accumulate in the proximal renal tubule (Barsoum *et al.*, 1984; Hard and Snowden, 1991; Luz and Murray, 1991).

Spleen: The incidences of hematopoietic cell proliferation in the red pulp of the spleen in all exposed groups of males were significantly greater than in the controls (Tables 19 and D5), and the severity of this lesion increased with increasing exposure concentration. Hematopoiesis is a process that normally

occurs to some extent in the spleen of $B6C3F_1$ mice; therefore, in this study, the diagnosis was generally made when the amount of hematopoietic cell proliferation exceeded an established threshold. This minimal change is consistent with that identified in the 13-week study and is considered related to administration of phenolphthalein.

Liver: The incidences of hepatocellular adenoma in all exposed groups of males and females and of hepatocellular adenoma or carcinoma (combined) in 6,000 and 12,000 ppm males and all exposed groups of females were significantly less than those in the controls, and the incidences of these lesions occurred with significant negative trends (Tables 20, C3, and D3). The incidences of hepatocellular adenoma or carcinoma (combined) in all control and exposed groups were within the ranges in historical controls in NTP 2-year feed studies (Tables C4c and D4d). Multiple hepatocellular adenomas were observed more frequently in the control groups than in the exposed groups (Tables C1 and D1). The incidences of clear cell and eosinophilic foci in all exposed groups of males and of mixed cell foci in 12,000 ppm males were significantly less than those in the controls (Table C5). The incidences of eosinophilic foci in exposed groups of females were significantly less than that in the controls (Table D5). Foci of cellular alteration, adenomas, and carcinomas generally represent a spectrum in the progression of neoplasia in the liver of $B6C3F_1$ mice. Although there is a known association of the incidences of these neoplasms with body weight in the $B6C3F_1$ mouse, the slightly lower mean body weights of exposed groups could not account for the decreased incidences observed in this study. These decreases were considered related to the administration of phenolphthalein.

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Feed Study of Phenolphthalein

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male				
Number Examined Microscopically	50	50	50	49
Clear Cell Focus[a]	24	6**	1**	0**
Eosinophilic Focus	22	6**	1**	1**
Mixed Cell Focus	6	2	1	0*
Hepatocellular Adenoma, Multiple	12	1	0	1
Hepatocellular Adenoma (Includes Multiple)				
Overall rate[b]	22/50 (44%)	12/50 (24%)	8/50 (16%)	10/49 (20%)
Adjusted rate[c]	53.5%	29.3%	19.6%	25.0%
Terminal rate[d]	21/40 (53%)	6/33 (18%)	5/36 (14%)	7/36 (19%)
First incidence (days)	582	563	495	522
Logistic regression test[e]	P=0.010N	P=0.032N	P=0.003N	P=0.011N
Hepatocellular Carcinoma (Includes Multiple)				
Overall rate	8/50 (16%)	10/50 (20%)	5/50 (10%)	9/49 (18%)
Adjusted rate	19.5%	25.0%	11.8%	22.0%
Terminal rate	7/40 (18%)	5/33 (15%)	2/36 (6%)	5/36 (14%)
First incidence (days)	727	560	500	639
Logistic regression test	P=0.533	P=0.385	P=0.273N	P=0.474
Hepatocellular Adenoma or Carcinoma[f]				
Overall rate	27/50 (54%)	20/50 (40%)	11/50 (22%)	16/49 (33%)
Adjusted rate	64.2%	45.3%	25.8%	38.4%
Terminal rate	25/40 (63%)	10/33 (30%)	6/36 (17%)	11/36 (31%)
First incidence (days)	582	560	495	522
Logistic regression test	P=0.016N	P=0.123N	P=0.001N	P=0.026N

(continued)

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Female				
Number Examined Microscopically	50	50	50	50
Clear Cell Focus	1	2	0	0
Eosinophilic Focus	20	4**	2**	1**
Mixed Cell Focus	3	2	1	0
Hepatocellular Adenoma, Multiple	5	0	0	0
Hepatocellular Adenoma (Includes Multiple)				
Overall rate	17/50 (34%)	2/50 (4%)	6/50 (12%)	1/50 (2%)
Adjusted rate	41.2%	6.5%	17.1%	3.6%
Terminal rate	15/39 (38%)	2/31 (6%)	5/34 (15%)	1/28 (4%)
First incidence (days)	597	737 (T)	693	737 (T)
Logistic regression test	P<0.001N	P<0.001N	P=0.015N	P<0.001N
Hepatocellular Carcinoma				
Overall rate	6/50 (12%)	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adjusted rate	14.9%	3.2%	0.0%	3.6%
Terminal rate	5/39 (13%)	1/31 (3%)	0/34 (0%)	1/28 (4%)
First incidence (days)	708	737 (T)	—[g]	737 (T)
Logistic regression test	P=0.042N	P=0.099N	P=0.025N	P=0.111N
Hepatocellular Adenoma or Carcinoma[h]				
Overall rate	21/50 (42%)	3/50 (6%)	6/50 (12%)	2/50 (4%)
Adjusted rate	49.8%	9.7%	17.1%	7.1%
Terminal rate	18/39 (46%)	3/31 (10%)	5/34 (15%)	2/28 (7%)
First incidence (days)	597	737 (T)	693	737 (T)
Logistic regression test	P<0.001N	P<0.001N	P=0.002N	P<0.001N

(T)Terminal sacrifice
* Significantly different (P≤0.05) from the control group by the logistic regression test
** P≤0.01
[a] Number of animals with lesion
[b] Number of animals with neoplasm per number of animals with liver examined microscopically
[c] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[d] Observed incidence in animals surviving until the end of the study
[e] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in an exposure group is indicated by N.
[f] Historical incidence for 2-year NTP feed studies with untreated controls (mean ± standard deviation): 596/1,465 (40.7% ± 14.5%); range, 10%-68%
[g] Not applicable; no neoplasms in animal group
[h] Historical incidence: 313/1,464 (21.4% ± 13.0%); range, 3%-56%

Tooth: There were decreased incidences of degeneration of the teeth in all exposed groups of male mice (0 ppm, 22/49; 3,000 ppm, 12/50; 6,000 ppm, 4/50; 12,000 ppm, 1/49; Table C5) Degeneration of the teeth is a common spontaneous lesion in male B6C3F$_1$ mice and is characterized by deformity and distortion of dental profiles, excessive dentine formation, and hyperplasia of various cellular elements associated with tooth formation. Because this lesion occurs more frequently in males, it is possible that male hormones play a role in its development or that female hormones are protective. The decrease in the incidence of this lesion was considered to be related to phenolphthalein administration.

Pituitary gland: The incidences of hyperplasia of the pars distalis of the pituitary gland in 6,000 and 12,000 ppm females were significantly less than that in the controls (0 ppm, 11/49; 3,000 ppm, 4/50; 6,000 ppm, 1/49; 12,000 ppm, 1/48; Table D5). Because of the central role the pituitary gland in hormonal homeostasis, it is probable that this decrease in the incidence of hyperplasia is related to the administration of phenolphthalein. However, its significance and mechanism of relationship to phenolphthalein administration are not understood.

Thyroid gland: There were decreased incidences of follicular cell hyperplasia of the thyroid gland in all exposed groups of females (27/50, 8/50, 3/50, 7/50; Table D5). However, all hyperplasias observed in the control mice were of minimal severity. As with the pituitary gland, this change is of questionable biological significance, but is also probably related to exposure to phenolphthalein.

GENETIC TOXICOLOGY

Phenolphthalein, tested independently in two laboratories, was not mutagenic in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without induced rat or hamster liver S9 metabolic activation enzymes (Mortelmans *et al.*, 1986; Table E1). In cytogenetic tests with cultured Chinese hamster ovary cells, no induction of sister chromatid exchanges was observed after treatment with 0.5 to 50 μg/mL phenolphthalein with or without S9 (Table E2). Cytotoxicity was noted at the 50 μg/mL dose level, and culture times were extended to maximize the proportion of cells available for analysis at this dose level. Results of the *in vitro* chromosomal aberrations test with phenolphthalein were positive for the two trials conducted with S9; no increase in chromosomal aberrations was observed in the absence of S9 (Witt *et al.*, 1995; Table E3). At the 50 μg/mL dose level, approximately one quarter of all cells contained aberrations, and most of these were chromosome breaks located at the distal end of X_q. The significance of this preferential breakage is as yet unknown, but it has been noted with other chemicals, and it occurs only in the presence of S9. A discussion of possible mechanisms for this phenomenon is presented in Galloway *et al.* (1987). Results from the *in vivo* mouse peripheral blood micronucleus test were positive for both male and female mice (Dietz *et al.*, 1992; Table E4). Significant increases in micronuclei were noted for both the normochromatic and polychromatic erythrocyte populations. The increases in micronucleated normochromatic erythrocytes, which indicate the effects of chronic exposure, occurred at all exposure concentrations in males and females. The effects on polychromatic erythrocytes, which indicate chromosomal damage induced within the 48 hours preceding analysis, occurred only at 25,000 and 50,000 ppm, the two highest exposure concentrations.

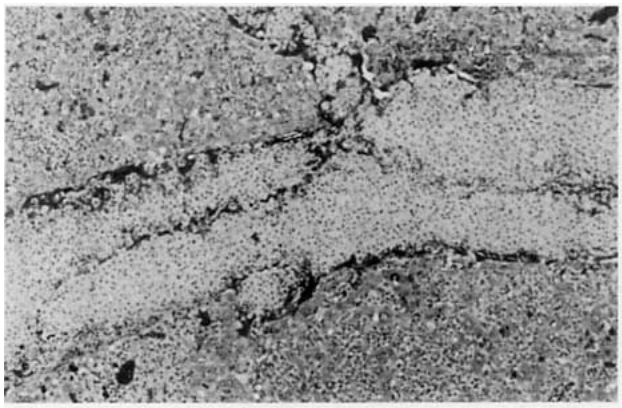


PLATE 1
Histiocytic sarcoma of the liver of a male B6C3F$_1$ mouse exposed to 12,000 ppm phenolphthalein in feed for 2 years. Note the thick band of neoplastic histiocytes within the liver. H&E; 50×

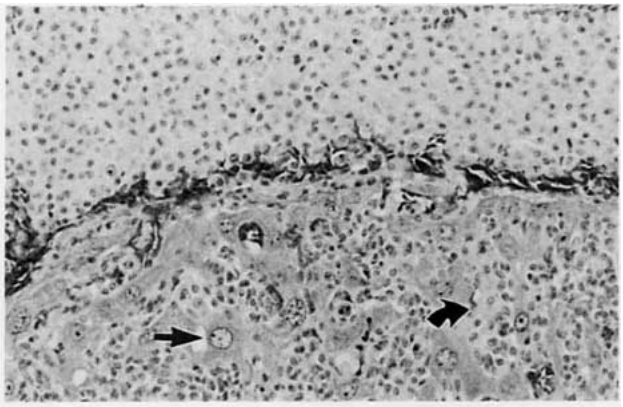


PLATE 2
A higher magnification of the histiocytic sarcoma shown in Plate 1. The upper half demonstrates the variability in nuclear shape and the abundant cytoplasm present in the neoplastic cells. In the lower half, neoplastic cells (large arrow) are present within expanded hepatic sinusoids. Also note few remaining hepatocytes (small arrow). H&E; 130×

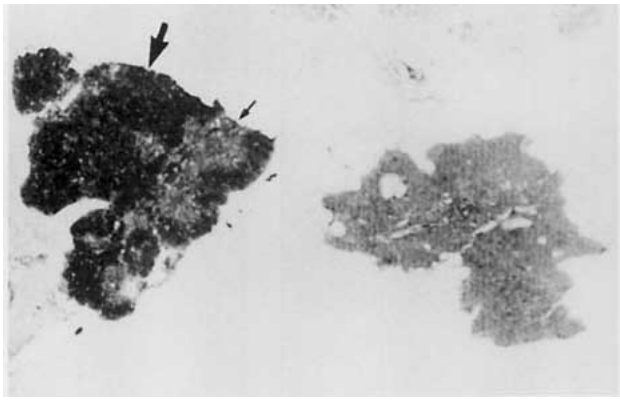


PLATE 3
Thymus of a male B6C3F$_1$ mouse exposed to 12,000 ppm phenolphthalein in feed for 2 years. The section to the left is normal thymic tissue with a darkly stained peripheral cortex (large arrow) and an inner medullary area (small arrow). The section to the right is abnormal without a distinct cortex or medulla. H&E; 10×

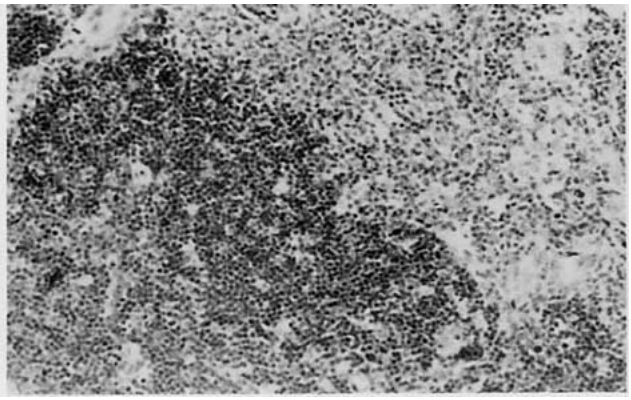


PLATE 4
Normal thymus of a control male B6C3F$_1$ mouse in the 2-year feed study of phenolphthalein. The cortex is to the left and the medulla in the upper right. H&E; 130×

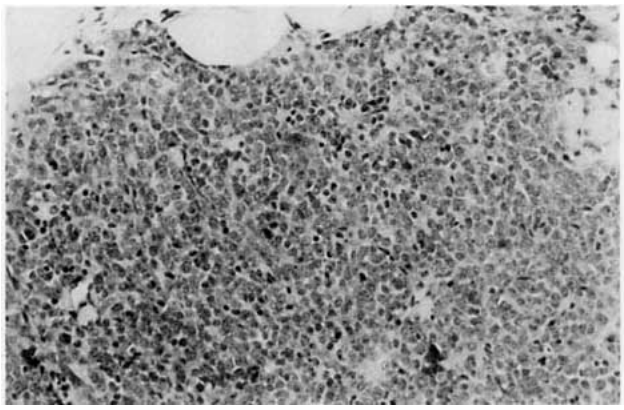


PLATE 5
Higher magnification of Plate 3. Note the population of large lymphocytes with rounded to oval nuclei and scant cytoplasm. Compare to normal thymic tissue in Plate 4. H&E; 130×

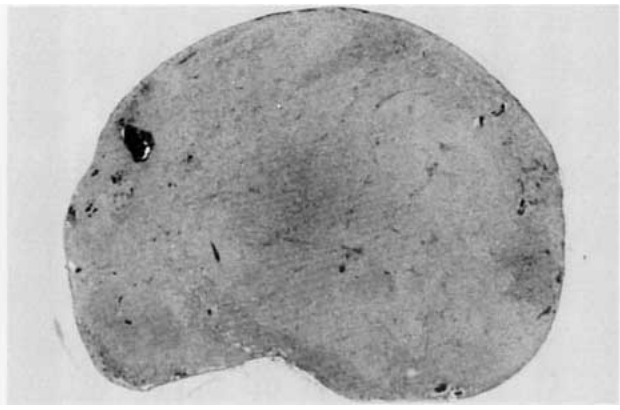


PLATE 6
Ovary of a female $B6C3F_1$ mouse exposed to 12,000 ppm phenolphthalein in feed for 2 years. Note the expansile mass composed of a homogeneous sheet of cells within and expanding the ovary. Compare with normal ovary in Plate 7 at much higher magnification. H&E; 6×

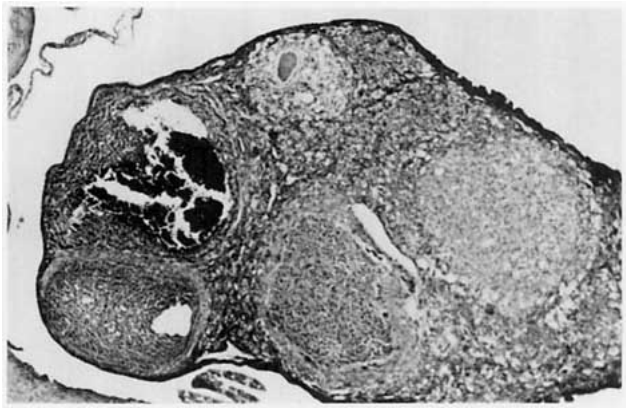


PLATE 7
Relatively normal ovary of a control female $B6C3F_1$ mouse in the 2-year feed study of phenolphthalein. H&E; 20×

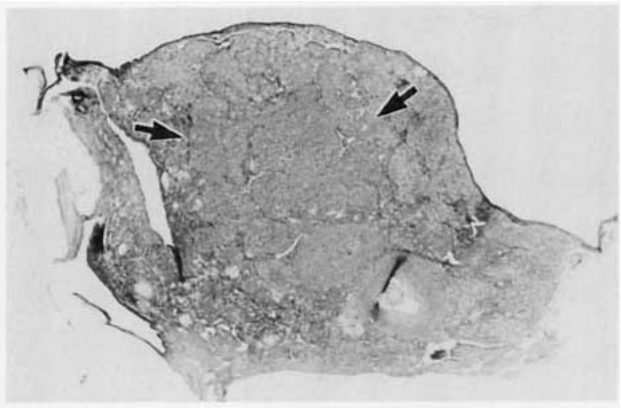


PLATE 8
Ovary of a female $B6C3F_1$ mouse exposed to 6,000 ppm phenolphthalein in feed for 2 years. Note the area of hyperplasia (arrows) within the ovary. H&E; 20×

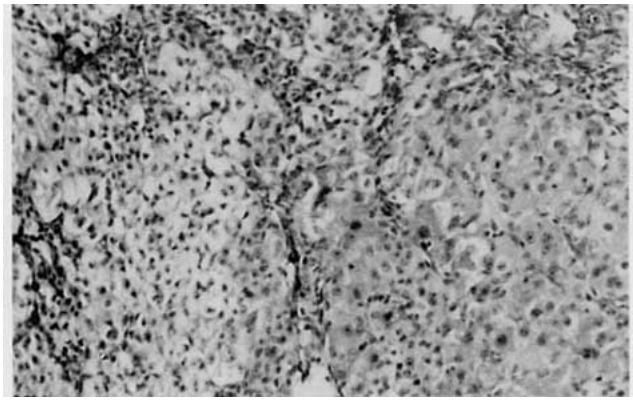


PLATE 9
A higher magnification of Plate 8. Note the component cells with abundant finely granular (right) or vesiculated (left) cytoplasm. H&E; 130×

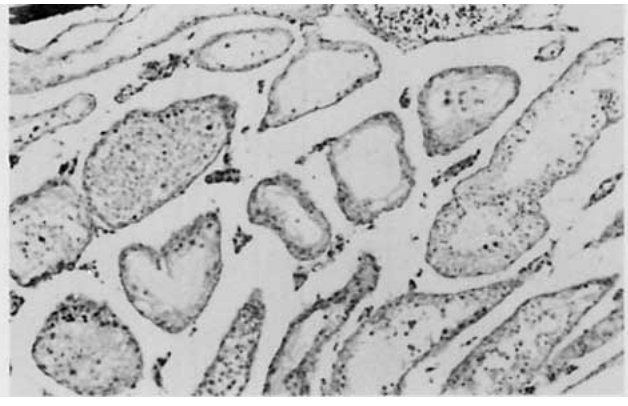


PLATE 10
Marked degeneration of the seminiferous tubules in the testis of a male B6C3F$_1$ mouse exposed to 12,000 ppm phenolphthalein in feed for 2 years. Note the variable loss of germinal epithelium within tubules. Compare to a normal testis in Plate 11. H&E; 65×

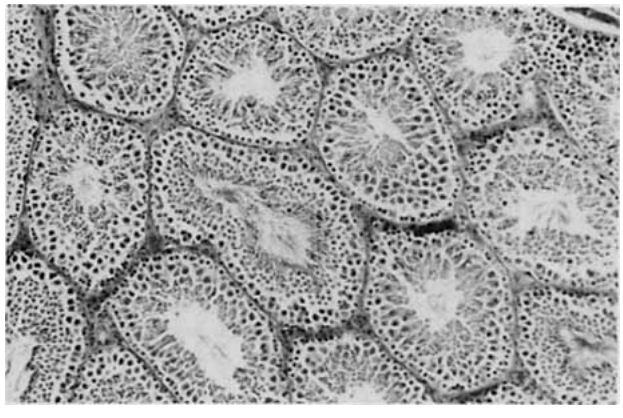


PLATE 11
Testis of a control male B6C3F$_1$ mouse in the 2-year feed study of phenolphthalein. Note normal appearing germinal epithelium. H&E; 65×

DISCUSSION AND CONCLUSIONS

Phenolphthalein was nominated by the National Cancer Institute for toxicity and carcinogenicity studies because it is an over-the-counter laxative for which no carcinogenesis bioassay has been reported in the literature. Phenolphthalein is usually taken as a 60 mg oral dose, four times a day, for an approximate daily dose of 4 to 5 mg/kg. The probable mechanism for the laxative effect is stimulation of the mucosal nerve plexus in the colon, combined with water and electrolyte secretion or inhibition of water absorption in both large and small intestines, and decreased glucose absorption (Pietrusko, 1977; Saunders *et al.*, 1978; *Goodman and Gilman's*, 1990).

In the 13-week feed studies, rats and mice were exposed to phenolphthalein at concentrations up to 50,000 ppm. All rats and mice survived to the end of the studies. No chemical-related clinical signs of toxicity were observed in rats or mice, and there was no evidence of a laxative effect in these animals. In the 13-week studies, there were no chemical-related gross or microscopic lesions in rats. However, hypoplasia of the bone marrow occurred in male and female mice exposed to 12,000 ppm or greater. This lesion was characterized by decreased amounts of hematopoietic tissue with a decreased myeloid to erythroid ratio. Splenic hematopoiesis was observed in male mice exposed to 25,000 or 50,000 ppm. There were also effects on the male reproductive system in mice. There was no evidence of reproductive toxicity in female B6C3F$_1$ mice or in male or female F344/N rats. Lower epididymal weights and sperm density and an increased incidence of abnormal sperm were observed in male mice at all exposure concentrations evaluated (12,000, 25,000, and 50,000 ppm). In some seminiferous tubules one or more generations of germ cells in portions of or in the entire tubule were missing. Often such tubules were surrounded by seminiferous tubules that looked normal. This suggested an intermittent commitment of spermatogonia to differentiation, producing a "blank space" in the progression of cells through spermatogenesis. The lesion may be mimicked by dosing with a toxicant for a limited period of time followed by quick recovery of the testis, as has been observed in rats administered 2-methoxyethanol in drinking water (NTP, 1993a). However, this is the only instance in the history of the NTP where continued exposure to a chemical has apparently caused stops and starts in spermatogenesis.

In order to further analyze the reproductive toxic effects in mice, a continuous breeding study of phenolphthalein was conducted in Swiss (CD-1®) mice according to a protocol previously described (Lamb, 1985; Heindel *et al.*, 1989). In this study, male and female mice were given 1,000, 7,000, or 30,000 ppm phenolphthalein in feed for a 98-day cohabitation period, and fertility during this period was assessed (Appendix N; NTP, 1991). Phenolphthalein caused lower fertility in the 7,000 and 30,000 ppm groups. Overall, the mean numbers of litters per pair in the 7,000 and 30,000 ppm groups were 24% and 50% lower than in the controls. The numbers of live pups per litter in these groups were 58% and 59% lower than in the controls. There was also postnatal toxicity in the F$_1$ generation, as indicated by a 30% to 71% lower survival than that of the controls (all of the deaths occurred during the first 4 days of life).

Phenolphthalein can cross the placental barrier in mice (strain not specified) (Visek *et al.*, 1956). The lower survival in the F$_1$ generation observed in this continuous breeding study in Swiss (CD-1®) mice may be attributed to effects to the developing fetus from *in utero* exposure, postnatal exposure via the milk, or from a combination of these exposures. No reproductive or teratogenic effects were observed in mice (strain not specified) exposed to 250 mg phenolphthalein/kg body weight per day in feed (Stockinger, 1965), but this study was conducted at lower doses than those used in the continuous breeding study. In the continuous breeding study, the exposure concentrations of 7,000 and 30,000 ppm were equivalent to daily doses of approximately 1,056 and 4,530 mg/kg, respectively.

In a crossover mating trial with Swiss (CD-1®) mice (control males mated to 7,000 ppm females or control females mated to 7,000 ppm males), it was found that the lower fertility, as measured by a lower number of pups born per litter, resulted from effects in the female mice. It should be noted that the 7,000 ppm male Swiss (CD-1®) mice exhibited approximately a 20% lower sperm count relative to the controls, but the lower sperm count did not affect fertility. The relationship between lower sperm counts and fertility in a number of species is an area of continuing investigation (Medical Research Council, 1995).

In summary, phenolphthalein produced significant reproductive toxicity in both Swiss (CD-1®) and $B6C3F_1$ mice as measured by lower epididymal weight and sperm density. Exposed female Swiss (CD-1®) mice mated to control male Swiss (CD-1®) mice had fewer than half the number of live pups per litter as controls.

The mechanism for the reproductive toxicity observed in mice (but not in rats) is not fully understood. Phenolphthalein contains a triphenylmethane structure, and this structure may act as a nonsteroidal estrogen agonist or antagonist through interaction with the estrogen receptor protein (Ravdin *et al.*, 1987). Studies by other groups are underway to assess whether or not environmental estrogenic compounds play a significant role in reproductive disorders in humans (Jobling *et al.*, 1995; Medical Research Council, 1995).

In the 2-year feed studies of phenolphthalein, there were no chemical-related effects on survival in male or female rats or male mice; there was marginally lower survival in the 12,000 ppm female mice. No chemical-related clinical signs were observed.

Mean body weights of exposed groups of rats were 5% to 10% lower than those of the controls during most of the study, but toward the end of the study, the body weight differences became more pronounced. The body weight differences in mice were less than those in rats.

Feed consumption by exposed groups was similar to that by the controls. Comparisons of doses based on body surface area are provided in Table 21.

At the end of the 2-year study, organ toxicity and/or carcinogenic effects were observed in the adrenal gland of rats. These effects developed late in the study, and no toxicity was observed in these organs in the 13-week rat study.

The incidences of benign pheochromocytoma of the adrenal medulla were significantly increased in all groups of exposed male rats and in 12,000 ppm female rats. The incidences of benign pheochromocytoma in all groups of exposed male rats and in 12,000 and 25,000 ppm female rats exceeded the ranges in historical controls in NTP 2-year feed studies. The incidence of benign or malignant pheochromocytoma (combined) of the adrenal medulla in 12,000 and 25,000 ppm female rats was significantly greater than that of the controls and exceeded the range in historical controls in NTP 2-year feed studies. In the NTP database of approximately 450 chemicals, a chemical-induced neoplasm response is observed in the adrenal gland of rats two to three times more frequently than in mice. These increases in the incidences of pheochromocytoma of the adrenal medulla were considered to be related to exposure to phenolphthalein because the effect was observed in males and females, and in the groups cited above the neoplasm rates were greater than those of the concurrent and historical control rates. However, increased incidences were not observed in 50,000 ppm female rats.

The incidence of chronic nephropathy, which is common in aging rats (particularly males), was increased in exposed groups of female rats, and the severity of nephropathy was increased in exposed groups of male rats and slightly increased in exposed groups of female rats. In males, changes secondary to the exacerbated renal disease and indicative of compromised renal function (hyperplasia of the parathyroid gland, fibrous osteodystrophy of the bone, and mineralization and degeneration of the glandular stomach) were also increased, but not in a dose-related manner. Additionally, there were increases in the incidences of hyperplasia and neoplasms of the renal tubule epithelium. These lesions were confirmed by an extended evaluation of the kidneys. No mechanism by which these renal proliferative lesions developed was readily apparent, nor were these studies designed to determine a mechanism. One possible mode of action is consistent

TABLE 21
Summary of Feed and Compound Consumption by Rats and Mice in the 2-Year Feed Studies of Phenolphthalein

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male Rats				
Mean for Weeks 1-13				
(g feed/day)	16.3	16.2	17.0	16.6
(mg/kg per day)	0	789	1,714	3,375
(mg/m^2 per day)[a]	0	4,102	8,912	17,550
Mean for Weeks 53-104				
(g feed/day)	14.4	15.3	15.9	15.8
(mg/kg per day)	0	434	953	1,880
Female Rats				
Mean for Weeks 1-13				
(g feed/day)	10.7	10.4	10.4	11.0
(mg/kg per day)	0	787	1,655	3,423
(mg/m^2 per day)	0	4,092	8,603	17,799
Mean for Weeks 53-105				
(g feed/day)	11.4	11.3	11.4	11.8
(mg/kg per day)	0	477	1,013	2,125

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male Mice				
Mean for Weeks 1-13				
(g feed/day)	4.3	4.3	4.4	4.2
(mg/kg per day)	0	453	940	1,802
(mg/m^2 per day)	0	1,359	2,820	5,406
Mean for Weeks 53-104				
(g feed/day)	4.8	4.8	4.9	4.8
(mg/kg per day)	0	291	589	1,181
Female Mice				
Mean for Weeks 1-13				
(g feed/day)	5.1	4.9	5.3	5.1
(mg/kg per day)	0	651	1,407	2,640
(mg/m^2 per day)	0	1,953	4,221	7,920
Mean for Weeks 53-105				
(g feed/day)	5.3	5.3	5.2	5.2
(mg/kg per day)	0	311	617	1,255

[a] Calculation for body surface area dose based on Freireich *et al.*, 1966; $mg/m^2 = K_m \times$ (dose in mg/kg), where K_m is 37 for humans, 5.2 for rats, and 3.0 for mice. (K_m is a conversion based on average height-to-body weight ratio.) Compound consumption values for humans = 5 mg/kg and 185 mg/m^2.

with the theory of increased cell replication providing a "fertile ground" for increased mutation rates and neoplasm development. To a certain point, increased kidney damage is thought to increase the amount of renal tubule epithelial regeneration via cell replication. In one study, [^{3}H]-thymidine labeling demonstrated increased levels of DNA synthesis to be directly proportional to increased severity of nephropathy in aging female F344/NCr rats (Konishi and Ward, 1989).

The findings in the male rat kidney are similar to those observed with other chemicals including quercetin (NTP, 1992), coumarin (NTP, 1993b), and 3,4-dihydrocoumarin (NTP, 1993c). With these chemicals, there was no evidence for toxicity to the kidney in the 13-week studies, but toward the end of the 2-year studies, the severity of nephropathy increased in male rats (and to a lesser extent in female rats), and there were a few kidney neoplasms in male rats. The greater sensitivity of the male rat to this kidney toxicity is apparently due to a greater susceptibility of male rats to spontaneous nephropathy during aging and the exacerbation of this disease by chemical administration. Changes in glomerular permeability, resulting in proteinuria, progressive glomerular sclerosis, tubule damage, inflammation, and interstitial fibrosis, are associated with the process of aging in rats.

There was a decrease in the incidence of mammary gland fibroadenomas in groups of exposed female rats. Previous studies have shown that decreases in the incidences of some naturally occurring benign neoplasms, such as mammary gland fibroadenoma, are associated with lower body weights relative to those of the controls (Rao *et al.*, 1987; Seilkop, 1995). The lower incidences of female rat mammary gland neoplasms in this study were also thought to be related to the lower body weights.

At the end of the 2-year mouse study, carcinogenic effects were observed in the hematopoietic system of males and females and the ovary of females. There were increases in the incidences of histiocytic sarcomas and malignant lymphomas of thymic origin in all exposed groups of male and female mice. These effects were clearly related to exposure to phenolphthalein. The incidences of histiocytic sarcoma in all groups of exposed male and female mice exceeded the ranges in historical controls in NTP 2-year feed studies. The incidences of malignant lymphoma (all types) in all exposed groups of female mice also exceeded the ranges in historical controls in NTP 2-year feed studies.

Chemical-associated increases in the incidences of histiocytic sarcoma are uncommon in NTP studies; however, increased incidences have been observed in the studies with 1,3-butadiene (NTP, 1984b, 1993d) (an increase in thymic lymphomas also occurred) and tetrafluoroethylene (NTP, 1996a). Spontaneous histiocytic sarcoma occurs two to three times more frequently in female mice than in male mice. Similarly, in the tetrafluoroethylene mouse study, incidences in groups of females were about twice those observed in groups of males. The reverse was true in the current study. Histiocytic sarcomas occur most commonly in the liver, but are often widespread and involve numerous tissues. In the current study, histiocytic sarcoma was identified in the liver of all but three mice diagnosed with histiocytic sarcoma. Histiocytic sarcomas are generally considered to arise from a macrophage/histiocyte including the specialized Kupffer cell of the liver, but definitive data relative to the site of origin are lacking. In studies where histiocytic sarcomas were transplanted, the local lesions generally remained small, while the liver became quite large (Frith *et al.*, 1980). There is also evidence that many of these neoplasms produce excessive amounts of lysozyme, which can result in the accumulation of hyaline droplets in the proximal renal tubules as apparently occurred in the current study.

Exposure to phenolphthalein was associated with a clear increase in the incidence of malignant lymphoma (all types) in groups of exposed female mice. However, significant increased incidences of proliferative lymphocytic lesions that arose within the thymus were observed in both male and female mice. A number of classification schemes for malignant lymphomas (Dunn, 1954; Pattengale and Taylor, 1983; Wogan, 1984), primarily based upon histomorphology, have been proposed. However, it is often difficult to categorize consistently the various lymphomas. Further, as more is learned, it appears that there is little biological relevance for some of the classification schemes. Therefore, in most recent NTP studies, all lymphomas have been combined

under the diagnosis of malignant lymphoma. However, under certain circumstances, subclassification of lymphomas may be necessary to better understand the effects associated with an administered agent. In the $B6C3F_1$ mouse, the vast majority of spontaneous lymphomas arise from within the spleen or lymph nodes and are of B-cell origin. Lymphomas of the T-cells are much less common, and lymphomas arising from the thymus are uncommon. With phenolphthalein, 1,3-butadiene, and 2′,3′-dideoxycytidine (ddC) (Sanders *et al.*, 1995), the lymphomas originated primarily in the thymus. In this study, many proliferative lesions (atypical hyperplasias and lymphomas) clearly arose within the thymus of exposed males and females. Lymphomas were considered of thymic origin when they were observed only in the thymus or in the thymus and metastatic only to other sites within the chest cavity. Additionally, there was an increase in the incidence of atypical hyperplasia of the thymus in exposed males and females. It was apparent that, in general, the proliferative lesions of the thymus represented a morphological continuum in which the latter stage is malignant lymphoma. While there is a high probability that the lesions diagnosed as lymphomas are malignant neoplasms, there is less certainty as to the biological behavior of the smaller lesions (atypical hyperplasia). These smaller proliferative lesions are at least pre-neoplastic lesions and at most an early lymphoma. Many of the mice in this study had advanced lymphoma involving multiple tissues and organs. In those mice, there was less confidence in determining the site of origin; however, because of the numerous thymic lesions observed in this study, it was expected that a number of those widespread lymphomas were of thymic origin. Proliferative lesions (atypical hyperplasia and malignant lymphoma) of the thymus in exposed males and females were considered related to phenolphthalein exposure.

Phenolphthalein, 1,3-butadiene, and ddC are genotoxic in *in vivo* tests, suggesting that genotoxicity may be a contributing factor to the observation of thymic lymphomas. However, further work is in progress to see if phenolphthalein accumulates in the thymus and to identify possible mechanisms. Although mutations that activate *ras* proto-oncogenes are common in thymic lymphomas induced by a number of environmental agents including *N*-methylnitrosurea (Warren *et al.*, 1990; Corominas *et al.*, 1991) and radiation (Janowski *et al.*, 1990), *ras* mutations have not been commonly observed in the ddC-induced thymic lymphomas (Wiseman *et al.*, 1994) and were observed in only 2 of 11 thymic lymphomas from mice exposed to 1,3-butadiene (Goodrow *et al.*, 1994). *Ras* mutations were more common in 1,3-butadiene-induced lung and liver tumors than in the 1,3-butadiene-induced thymic lymphomas examined (6/7 lung tumors had K-*ras* mutations; 3/7 liver tumors had K-*ras* mutations; and 4/7 liver tumors had H-*ras* mutations) (Goodrow *et al.*, 1994).

In mice, exposure to phenolphthalein increased the incidence of ovarian hyperplasia in 3,000 and 12,000 ppm females and ovarian neoplasms in all groups of female mice. Generally, ovarian neoplasms fall into three categories: 1) epithelial, 2) germ cell, or 3) sex-cord stromal (e.g., granulosa cell tumors and luteomas). In the $B6C3F_1$ mouse, ovarian neoplasms are uncommon; epithelial cell tumors are slightly more common than granulosa cell tumors in control females. The morphology of the proliferative lesions of the ovary in the female mice in this study was somewhat distinctive from those typically observed in controls. It was apparent that cells composing proliferative lesions were often luteinized and were of sex-cord stromal origin. However, it was uncertain if component cells were granulosa, thecal, interstitial, or a combination. Non-specific categorization of these ovarian neoplasms as sex-cord stromal reflects that uncertainty.

Ovarian tumors (granulosal and epithelial) have previously been reported in mice in NTP studies after administration of eight other chemicals: benzene (NTP, 1986a), 1,3-butadiene (NTP, 1984b, 1993d), *N*-methylolacrylamide (NTP, 1989a), 5-nitroacenaphthene (NCI, 1978), nitrofurantoin (NTP, 1989b), nitrofurazone (NTP, 1988), 4-vinylcyclohexene (NTP, 1986b), and 4-vinyl-1-cyclohexene diepoxide (NTP, 1989c). The mechanism for the induction of ovarian tumors with some of these chemicals may involve atrophy of the ovary and oocyte destruction followed by a subsequent decrease in estrogen production, which leads to a compensatory increase in pituitary gland gonadotropin release. The increased stimulation by gonadotropins is thought to stimulate cell proliferation in the ovary and to promote the eventual development of

tumors. In the current study, no atrophy was observed, and the tumors are not similar to the epithelial and granulosa cell tumors observed in the previous NTP studies. The observation that phenolphthalein interacts with estrogen receptors (Ravdin *et al.*, 1987) may be one means by which this chemical stimulates cellular proliferation of the ovary. It has previously been demonstrated that estradiol can stimulate ovary cells to proliferate (including granulosa and theca cells) (Rao *et al.*, 1978); phenolphthalein may mimic the action of this steroid hormone by interacting with estrogen receptors of ovary cells. Alternatively, phenolphthalein may act as an antiestrogen in the pituitary gland and lead to an increase in gonadotropin production.

No chemical-related ovarian tumors have been reported in rats in any of the NTP studies. In the phenolphthalein study in rats, there was no tumor response at this site. Therefore, in our model systems, the mouse is more sensitive to a chemical-induced ovarian tumor response than the rat.

Cancer of the ovary is the fourth most common cancer in American women. There were an estimated 26,600 new cases diagnosed in 1995 and 14,500 deaths from this disease (SEER, 1995). In humans, approximately 80% of the ovarian tumors are of epithelial origin; other ovarian tumors may be of sex-cord stromal origin. Like many cancers, ovarian cancer is a disease of aging, with almost half of new cases occurring in women age 65 or older (SEER, 1995). In these mouse studies, ovarian effects were not observed in the 13-week study but developed toward the end of the 2-year study. The earliest occurrence of ovarian tumors in mice was noted on day 668. In this study, and in mice in general, the ovarian tumors were not fatal.

Environmental factors that might contribute to ovarian cancer in humans have not been identified. It is known that the rate for ovarian cancer in American women is two to three times higher than that for women in other parts of the world (Parkin *et al.*, 1993). It is not known if this difference in the ovarian cancer rate is due to environmental or genetic factors.

Like phenolphthalein, which has been shown to bind competitively to the estrogen receptor in MCF-7 cells (Ravdin *et al.*, 1987), other chemicals tested in the NTP bioassay have also been shown to interfere with estrogen binding at the estrogen receptor and to have mitogenic effects on breast cancer cells (Jobling *et al.*, 1995). These include zearalenone (NTP, 1982a), 2,4-dichlorophenol (NTP, 1989d), and butyl benzyl phthalate (NTP, 1982b, 1996b). However, of these chemicals, only phenolphthalein was shown to cause ovarian neoplasms in mice. Thus, demonstration of a chemical-induced estrogenic response (as reported from *in vitro* and/or *in vivo* studies) does not necessarily correlate with ovarian neoplasm or mammary gland neoplasm response in the F344/N rat and B6C3F$_1$ mouse studies. Further, these NTP chemicals with estrogenic activity show different neoplasm patterns in other tissues. This suggests that phenolphthalein-induced neoplasms may be due to multiple factors, which might include specific metabolism and distribution of the chemical, estrogenic effects of the chemical, or genotoxic properties of the chemical.

Estrogenic activity is found with compounds having different chemical structures, including estradiol, zearalenone, coumestrol, *o,p'*-DDT, kepone, bisphenol A, and impurities in phenol red (Katzenellenbogen, 1995). More information is needed on the molecular details of the three-dimensional structure of the estrogen receptor and its interaction with ligands to understand how these varied chemicals interact with the receptor. Studies with phenol red (phenolsulfonphthalein) have shown that lipophilic impurities in this dye have more estrogenic activity than the parent compound (Bindal and Katzenellenbogen, 1988; Bindal *et al.*, 1988), suggesting that the lipophilicity of chemicals is an important property in reaching or binding to the estrogen receptor.

The incidences of germinal epithelial degeneration of the testis in all exposed groups of male mice were significantly greater than that in the controls. The lesion varied from involvement of a few to all seminiferous tubules. Within the tubules, there was variable loss of sperm and germinal epithelial cells

(spermatogonia, spermatocytes, and spermatids), often with only Sertoli cells remaining. In the most severe cases, the cross-sectional diameter of the testis was decreased. Although more severe, this lesion is consistent with that observed in the 13-week study.

There was an increased incidence of myelofibrosis of the bone marrow of 12,000 ppm male and female mice and an increased severity of this lesion in exposed female mice. This was characterized by focal to multifocal replacement of bone marrow elements by clusters of loosely arranged spindle cells. The finding that phenolphthalein affects the bone marrow cells of mice in both the 13-week and 2-year studies shows that, in both short-term and long-term exposures, the bone marrow is a target tissue. Fibro-osseous lesions are observed in the flat and long bones of a number of strains of mice as spontaneous and induced lesions, and these lesions are very common in female $B6C3F_1$ mice. Female sex hormones, especially estradiol, appear to play a major role in the spontaneous development of myelofibrosis. Estrogen has been reported to cause neutropenia, thrombocytopenia, decreased bone marrow cellularity, and increased incidences of splenic hematopoiesis in mice (Fried *et al.*, 1974; Adler and Trobaugh, 1978). Witt *et al.* (1995) have also shown that phenolphthalein can induce micronuclei in bone marrow erythrocytes of mice. Micronuclei are formed from acentric chromosomal fragments or whole chromosomes generated through a variety of mechanisms such as mitotic loss of acentric fragments, mechanical consequences of chromosomal breakage and exchange events resulting in abnormal anaphase separation, or mitotic loss of whole chromosomes due to centromere or spindle failures. Because phenolphthalein induced chromosomal aberrations in cultured Chinese hamster ovary cells, induction of chromosomal breakage may be one of the mechanisms for the observed alterations in mouse bone marrow following exposure to phenolphthalein.

The decreases in the incidences of liver neoplasms and nonneoplastic lesions and the decreases in the incidences of proliferative lesions of the pituitary gland in female mice, the thyroid gland in female mice, and the tooth of male mice were considered to be chemical related.

There was no indication that phenolphthalein caused an increase in the incidences of lesions of the colon or rectum in rats or mice. In one limited study conducted in Australia to identify risk factors for colorectal cancer in humans, there was no indication that phenolphthalein taken as a laxative caused any disease in the colon or rectum (Kune, 1993). This study did not examine the potential of phenolphthalein to cause toxic or carcinogenic effects at other sites.

After oral administration, phenolphthalein is absorbed by the small intestine and is conjugated in the liver to phenolphthalein glucuronide, which passes into the colon where it is deconjugated and the active compound, phenolphthalein, is released. In humans, the laxative response may occur after an oral dose of phenolphthalein or the phenolphthalein glucuronide (Anand *et al.*, 1994).

In the 2-year studies, the plasma concentrations of total phenolphthalein were similar for all exposure concentrations. There was no consistent difference in plasma concentrations of total phenolphthalein between males and females. The findings that the plasma concentrations of total phenolphthalein were approximately the same (approximately 100 to 200 μg/mL plasma in rats and mice) for the exposure concentrations used in the 2-year studies may explain why dose-response increases in the incidences of kidney neoplasms in male rats, histiocytic sarcoma in male and female mice, malignant lymphoma in female mice, or ovarian neoplasms in female mice did not occur. However, in the single-dose toxicokinetic studies of phenolphthalein, the elimination half-lives in mice were approximately half those of rats (Appendix O; NTP, 1994).

The use of phenolphthalein as a laxative began early in the twentieth century (von Vámossy, 1908), and systematic studies of plasma concentrations of phenolphthalein or its metabolites in humans have not been reported in the literature.

Phenolphthalein was negative in the *Salmonella* mutagenicity assay with and without metabolic activation, but was positive in an *in vitro* chromosomal aberrations test conducted with S9 metabolic

activation. In conjunction with the 13-week feed study in mice, blood samples were analyzed at the end of study for the presence of micronucleated erythrocytes, and these were found to be significantly increased in male and female mice at exposure concentrations of 6,000 ppm phenolphthalein or higher (Witt *et al.*, 1995). In feed studies in Swiss (CD-1®) mice, phenolphthalein was found to increase the frequency of micronucleated erythrocytes in mice receiving 1,000 ppm phenolphthalein in feed for 14 weeks (Witt *et al.*, 1995).

Metabolism appears to be needed for genotoxicity with phenolphthalein. This hypothesis is supported by the requirement for S9 metabolic activation enzymes for the positive response in the chromosomal aberrations test in cultured Chinese hamster ovary cells. However, the predominant type of chromosomal damage observed in cultured Chinese hamster ovary cells treated with phenolphthalein was X chromosome breakage, a phenomenon that is not well understood, has been noted with only a few chemicals, and is only observed in cell cultures treated in the presence of S9. Therefore, the significance of the cultured Chinese hamster ovary cell results to interpretation of the *in vivo* responses is unclear. It is possible that the genetic damage induced by phenolphthalein is limited to chromosomal breakage (phenolphthalein, if converted to a quinoid, induces free oxygen radicals) and that point mutations and/or small deletions, such as are detected in the *Salmonella* assay, as well as other types of genetic damage, are not induced. For example, phenolphthalein did not induce sister chromatid exchanges in cultured Chinese hamster ovary cells with or without S9. It is unusual for a chemical to induce chromosomal aberrations but not sister chromatid exchanges in cultured Chinese hamster ovary cells, and this pattern of activity further supports the specificity of genetic damage induced by phenolphthalein.

In summary, phenolphthalein caused toxicity to the reproductive system of male and female mice. Phenolphthalein also caused neoplasms and nonneoplastic lesions in various tissues and organs in rats and mice (Tables 24 and 25). The mechanisms for these effects are not known; however, phenolphthalein has been shown to have estrogenic activity *in vitro* (Ravdin *et al.*, 1987) and *in vivo* (Nieto *et al.*, 1990) and genotoxic activity *in vivo* (Witt *et al.*, 1995). As is shown in Figure 1, phenolphthalein is capable of being converted to a quinoid structure. Quinoids are highly reactive chemicals capable of reacting with sulfhydryl groups and amino groups and forming oxygen free radicals (Brunmark and Cadenas, 1989; Degen and Metzler, 1989; Thompson *et al.*, 1990). It is not known at what specific sites in the cell these reactions might occur. Specific microenvironment conditions (e.g., pH) might be conducive to the formation of the phenolphthalein quinoid. Multiple mechanisms may be involved in causing the toxic/carcinogenic responses to phenolphthalein in the rodent.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity** of phenolphthalein in male F344/N rats based on markedly increased incidences of benign pheochromocytomas of the adrenal medulla and of renal tubule adenomas and adenomas or carcinomas (combined). There was *some evidence of carcinogenic activity* of phenolphthalein in female F344/N rats based on the increased incidences of benign pheochromocytomas of the adrenal medulla in the 12,000 ppm group and of benign or malignant pheochromocytomas (combined) in the 12,000 and 25,000 ppm groups. There was *clear evidence of carcinogenic activity* of phenolphthalein in male B6C3F$_1$ mice based on increased incidences of histiocytic sarcomas and of malignant lymphomas of thymic origin. There was *clear evidence of carcinogenic activity* of phenolphthalein in female B6C3F$_1$ mice based on increased incidences of histiocytic sarcomas, malignant lymphomas of all types, lymphomas of thymic origin, and benign sex-cord stromal tumors of the ovary.

Exposure of rats to phenolphthalein in feed for 2 years resulted in increased incidences of focal hyperplasia of the adrenal medulla in males and in increased incidences and/or severity of nephropathy of the kidney in males and females. Exposure of mice to phenolphthalein in feed for 2 years resulted

in increased incidences of atypical hyperplasia of the thymus in males and females, degeneration of the germinal epithelium of the testis in males, and ovarian hyperplasia in females.

Exposure of mice to phenolphthalein in feed for 2 years resulted in decreased incidences of hepatocellular neoplasms and nonneoplastic lesions in males and females.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

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

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

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	15	13	16
Natural deaths	14	20	22	21
Survivors				
Died last week of the study			1	
Terminal sacrifice	21	15	14	13
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(48)	(49)	(49)	(48)
Intestine large, rectum	(48)	(49)	(50)	(50)
Intestine large, cecum	(44)	(41)	(44)	(40)
Intestine small, duodenum	(47)	(50)	(49)	(47)
Intestine small, jejunum	(47)	(41)	(47)	(42)
Intestine small, ileum	(44)	(40)	(40)	(41)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	1 (2%)	3 (6%)		1 (2%)
Hepatocellular adenoma, multiple				1 (2%)
Histiocytic sarcoma			1 (2%)	
Mesentery	(20)	(7)	(3)	(3)
Histiocytic sarcoma			1 (33%)	
Oral mucosa	(1)		(1)	
Pharyngeal, squamous cell carcinoma			1 (100%)	
Pharyngeal, squamous cell papilloma	1 (100%)			
Pancreas	(50)	(50)	(50)	(47)
Histiocytic sarcoma			1 (2%)	
Acinus, adenoma			2 (4%)	1 (2%)
Acinus, adenoma, multiple	1 (2%)			
Salivary glands	(50)	(49)	(49)	(49)
Stomach, forestomach	(50)	(50)	(50)	(49)
Stomach, glandular	(50)	(50)	(50)	(49)
Cardiovascular System				
Heart	(50)	(49)	(50)	(50)
Schwannoma malignant			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant	1 (2%)	1 (2%)		2 (4%)
Pheochromocytoma benign	14 (28%)	15 (30%)	15 (30%)	19 (38%)
Bilateral, pheochromocytoma malignant			1 (2%)	
Bilateral, pheochromocytoma benign	3 (6%)	19 (38%)	19 (38%)	15 (30%)
Islets, pancreatic	(50)	(50)	(50)	(48)
Adenoma	3 (6%)	2 (4%)		1 (2%)
Carcinoma	1 (2%)	2 (4%)	1 (2%)	2 (4%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Endocrine System (continued)				
Parathyroid gland	(41)	(48)	(49)	(46)
Adenoma		1 (2%)		
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	13 (26%)	11 (22%)	16 (32%)	11 (22%)
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(50)	(50)	(49)	(49)
Bilateral, C-cell, adenoma	1 (2%)			
Bilateral, follicular cell, adenoma		1 (2%)		
C-cell, adenoma	8 (16%)	2 (4%)	3 (6%)	4 (8%)
C-cell, carcinoma			1 (2%)	2 (4%)
Follicular cell, adenoma			2 (4%)	3 (6%)
Follicular cell, carcinoma	1 (2%)			
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(49)	(50)	(49)
Adenoma	4 (8%)	4 (8%)	4 (8%)	4 (8%)
Carcinoma		2 (4%)	2 (4%)	
Bilateral, adenoma	1 (2%)			
Bilateral, carcinoma		1 (2%)		
Prostate	(50)	(50)	(50)	(50)
Adenoma	2 (4%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	42 (84%)	42 (84%)	46 (92%)	45 (90%)
Interstitial cell, adenoma	4 (8%)	4 (8%)	3 (6%)	4 (8%)
Hematopoietic System				
Bone marrow	(48)	(50)	(50)	(49)
Lymph node	(17)	(22)	(27)	(20)
Mediastinal, carcinoma, metastatic, kidney				1 (5%)
Renal, carcinoma, metastatic, kidney				1 (5%)
Lymph node, mandibular	(50)	(49)	(49)	(49)
Lymph node, mesenteric	(50)	(50)	(47)	(48)
Histiocytic sarcoma			1 (2%)	
Spleen	(50)	(50)	(50)	(49)
Fibrosarcoma		1 (2%)		
Hemangioma			1 (2%)	
Histiocytic sarcoma			1 (2%)	
Thymus	(50)	(49)	(47)	(47)
Histiocytic sarcoma			1 (2%)	
Thymoma benign		1 (2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Integumentary System				
Mammary gland	(43)	(47)	(48)	(47)
Adenoma	1 (2%)		1 (2%)	
Fibroadenoma	5 (12%)	2 (4%)	2 (4%)	1 (2%)
Fibroadenoma, multiple	1 (2%)			
Skin	(49)	(50)	(50)	(50)
Basal cell adenoma	2 (4%)		1 (2%)	
Keratoacanthoma	4 (8%)	2 (4%)	3 (6%)	1 (2%)
Squamous cell carcinoma		1 (2%)		
Pinna, schwannoma malignant	1 (2%)			2 (4%)
Subcutaneous tissue, fibroma	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Subcutaneous tissue, fibroma, multiple	1 (2%)			
Subcutaneous tissue, histiocytic sarcoma			1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)			1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteosarcoma				1 (2%)
Skeletal muscle	(2)	(5)	(4)	(1)
Osteosarcoma			1 (25%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Cerebrum, oligodendroglioma malignant	1 (2%)			
Spinal cord	(2)	(3)	(3)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)		1 (2%)
Alveolar/bronchiolar carcinoma		1 (2%)		
Carcinoma, metastatic, kidney				1 (2%)
Chordoma, metastatic, uncertain primary site		1 (2%)		
Histiocytic sarcoma			1 (2%)	
Osteosarcoma, metastatic, bone				1 (2%)
Osteosarcoma, metastatic, skeletal muscle			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, oral mucosa			1 (2%)	
Special Senses System				
Zymbal's gland	(1)		(1)	(2)
Adenoma			1 (100%)	
Carcinoma	1 (100%)			2 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Liposarcoma	1 (2%)			
Renal tubule, adenoma		4 (8%)	2 (4%)	6 (12%)
Renal tubule, carcinoma		1 (2%)	1 (2%)	2 (4%)
Renal tubule, oncocytoma benign				1 (2%)
Transitional epithelium, carcinoma			1 (2%)	
Urinary bladder	(49)	(50)	(50)	(50)
Transitional epithelium, carcinoma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear	31 (62%)	27 (54%)	24 (48%)	29 (58%)
Mesothelioma malignant	2 (4%)	2 (4%)		2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	50	50	50
Total primary neoplasms	159	155	157	166
Total animals with benign neoplasms	47	50	49	50
Total benign neoplasms	117	116	122	121
Total animals with malignant neoplasms	35	36	31	33
Total malignant neoplasms	42	39	35	45
Total animals with metastatic neoplasms	1	2	2	4
Total metastatic neoplasms	3	14	2	7
Total animals with malignant neoplasms of uncertain primary site		1		

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

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

Number of Days on Study	2 3 8	3 3 0	4 0 7	4 8 6	4 9 0	4 9 8	5 0 6	5 2 0	5 2 9	5 3 4	5 5 3	5 6 1	5 7 1	5 7 7	5 9 8	6 0 1	6 0 8	6 1 4	6 1 5	6 1 7	6 4 3	6 4 9	6 7 1	6 8 5	7 0 4
Carcass ID Number	0 1 1	0 1 7	0 5 0	0 3 1	0 4 3	0 3 2	0 3 8	0 3 2	0 2 7	0 2 4	0 3 4	0 1 3	0 4 4	0 2 0	0 3 6	0 4 9	0 2 2	0 2 5	0 3 0	0 1 6	0 3 9	0 2 8	0 3 5	0 4 1	0 4 7
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	A	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A	+	A	A	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	A	+	+	A	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery			+	+					+			+			+			+		+			+	+
Mesothelioma malignant, metastatic, epididymis																									
Oral mucosa																+									
Pharyngeal, squamous cell papilloma																X									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma, multiple																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant		X																							
Pheochromocytoma benign																	X						X	X	
Bilateral, pheochromocytoma benign																									X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	M	+	M	M	M	+	+	+	+	+	+	+	+	M	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X				X									X			X			X			
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma													X								X			X	
Follicular cell, carcinoma									X																
General Body System																									
Tissue NOS									+																

+: 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 Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 0 8	7 1 5	7 2 4	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 4 5	0 4 0	0 1 8	0 4 8	0 0 1	0 0 3	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 0 9	0 1 0	0 1 2	0 1 4	0 1 5	0 1 9	0 2 1	0 2 3	0 2 6	0 2 9	0 3 3	0 3 7	0 4 2	0 4 6	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma				X																						1
Mesentery	+				+				+	+			+		+			+	+	+	+		+		M	20
Mesothelioma malignant, metastatic, epididymis																				X						1
Oral mucosa																										1
Pharyngeal, squamous cell papilloma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma, multiple																	X									1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma										X																1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign	X		X		X						X	X	X			X	X			X			X		X	14
Bilateral, pheochromocytoma benign						X																X				3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X							X					X										3
Carcinoma																				X						1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	M	41
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X			X								X			X		X		X			X			13
Pars intermedia, adenoma														X												1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, C-cell, adenoma						X																				1
C-cell, adenoma								X							X			X					X		X	8
Follicular cell, carcinoma																										1
General Body System																										
Tissue NOS																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	2 3 8	3 3 0	4 0 7	4 8 6	4 9 0	4 9 8	5 0 6	5 2 0	5 2 9	5 3 4	5 5 3	5 6 1	5 7 1	5 7 7	5 9 8	6 0 1	6 0 8	6 1 4	6 1 5	6 1 7	6 4 3	6 4 9	6 7 1	6 8 5	7 0 4
Carcass ID Number	0 1 1	0 1 7	0 5 0	0 3 1	0 4 3	0 3 2	0 3 8	0 0 2	0 2 7	0 2 4	0 3 4	0 1 3	0 4 4	0 2 0	0 3 6	0 4 9	0 2 2	0 2 5	0 3 0	0 1 6	0 3 9	0 2 8	0 3 5	0 4 1	0 4 7
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X		
Bilateral, adenoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis																									
Bilateral, interstitial cell, adenoma				X	X	X	X	X	X	X	X		X	X	X		X	X	X	X	X		X	X	X
Interstitial cell, adenoma																X						X			
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	A	+	+
Lymph node				+	+	+		+				+			+			+	+	+	+		+		+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	M	+	+	+	+	M	+	+	M	+	+	+	+	+	M	+	+	+	+	+	M	+	+
Adenoma																									
Fibroadenoma										X															X
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Basal cell adenoma																								X	
Keratoacanthoma																								X	
Pinna, schwannoma malignant																									
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibroma, multiple										X															
Subcutaneous tissue, schwannoma malignant																	X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle		+		+																					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cerebrum, oligodendroglioma malignant													X												
Peripheral nerve		+		+																					
Spinal cord		+		+																					

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 0 8	7 1 5	7 2 4	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 4 5	0 4 0	0 1 8	0 4 8	0 0 1	0 0 3	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 0 9	0 1 0	1 1 2	1 1 4	1 1 5	1 1 9	1 2 1	2 2 3	2 2 6	2 2 9	3 3 3	3 3 7	4 4 2	4 4 6	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma					X												X							X		4
Bilateral, adenoma																					X					1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																	X					X				2
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																				X						1
Bilateral, interstitial cell, adenoma	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	42
Interstitial cell, adenoma		X		X																						4
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node		+	+	+			+								+											17
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	43
Adenoma		X																								1
Fibroadenoma	X												X									X				5
Fibroadenoma, multiple										X																1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Basal cell adenoma												X														2
Keratoacanthoma							X	X										X								4
Pinna, schwannoma malignant											X															1
Subcutaneous tissue, fibroma	X				X																					2
Subcutaneous tissue, fibroma, multiple																										1
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cerebrum, oligodendroglioma malignant																										1
Peripheral nerve																										2
Spinal cord																										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	2 3 8	3 3 0	4 0 7	4 8 6	4 9 0	4 9 8	5 0 6	5 2 0	5 2 9	5 3 4	5 5 3	5 6 1	5 7 1	5 7 7	5 9 8	6 0 1	6 0 8	6 1 4	6 1 5	6 1 7	6 4 3	6 4 9	6 7 1	6 8 5	7 0 4
Carcass ID Number	0 1 1	0 1 7	0 5 0	0 3 1	0 4 3	0 3 2	0 3 8	0 0 2	0 2 7	0 2 4	0 3 4	0 1 3	0 4 4	0 2 0	0 3 6	0 4 9	0 2 2	0 2 5	0 3 0	0 1 6	0 3 9	0 2 8	0 3 5	0 4 1	0 4 7
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Mesothelioma malignant, metastatic, epididymis																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear				+																					
Zymbal's gland														+											
Carcinoma														X											
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liposarcoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Transitional epithelium, carcinoma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X	X	X		X		X	X	X			X	X	X	X		X	X		X		X
Mesothelioma malignant																									X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 0 8	7 1 5	7 2 4	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 4 5	0 4 0	0 1 8	0 4 8	0 0 1	0 0 3	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 0 9	0 1 0	1 1 2	1 1 4	1 1 5	1 1 9	1 2 1	2 2 3	2 2 6	2 3 9	3 3 3	3 3 7	4 2 2	4 4 6	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																				X						1
Mesothelioma malignant, metastatic, epididymis																				X						1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liposarcoma												X														1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Transitional epithelium, carcinoma															X											1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X	X			X	X		X	X	X	X	X	X		X	X				X			X	31
Mesothelioma malignant																				X						2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm

Number of Days on Study	4 5 8	4 7 0	4 8 5	5 3 1	5 3 8	5 4 3	5 5 8	5 6 1	5 6 1	5 7 7	5 9 1	5 9 2	5 9 4	6 1 0	6 1 4	6 1 8	6 2 4	6 2 6	6 3 2	6 3 3	6 3 6	6 3 8	6 4 6	6 5 0	6 7 0
Carcass ID Number	0 7 6	0 7 7	0 8 6	0 7 3	0 8 3	0 9 8	0 9 5	0 5 4	0 5 6	0 6 6	0 6 7	0 5 5	0 6 4	0 7 4	0 7 1	0 8 5	0 7 5	0 5 9	0 7 0	0 6 2	0 5 2	0 5 3	0 5 7	0 8 8	0 9 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	A	A	+	+	+	+	+	+	+	+	A	A	+	A	+	+	+	+	+	+	A
Serosa, mesothelioma malignant, metastatic, epididymis																							X		
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serosa, mesothelioma malignant, metastatic, epididymis																							X		
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	A	+	+	+	A	+	A	+	+	+	A
Serosa, mesothelioma malignant, metastatic, epididymis																							X		
Intestine small, ileum	+	+	+	+	+	A	A	+	+	+	+	+	A	+	A	+	+	+	A	+	+	+	+	+	A
Serosa, mesothelioma malignant, metastatic, epididymis																							X		
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma										X				X											
Mesentery		+	+	+							+					+						+		
Mesothelioma malignant, metastatic, epididymis																							X		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis																							X		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serosa, mesothelioma malignant, metastatic, epididymis																							X		
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant											X														
Pheochromocytoma benign			X		X				X						X	X				X	X			X	
Bilateral, pheochromocytoma benign										X		X	X				X	X				X			
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																							X		
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenoma																				X					
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma														X	X										
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, follicular cell, adenoma																							X		
C-cell, adenoma				X																					

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm (continued)

Number of Days on Study	6 7 9	6 8 5	6 9 1	6 9 3	6 9 4	7 0 1	7 1 0	7 1 5	7 2 3	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 5 1	0 6 1	0 8 9	0 7 9	0 9 6	0 9 4	0 6 8	0 7 2	0 7 8	0 7 0	0 9 5	0 5 8	0 6 0	0 6 3	0 6 5	0 6 9	0 8 0	0 8 1	0 8 2	0 8 4	0 8 7	0 9 1	0 9 3	0 9 7	0 9 9	1 0 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	A	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Serosa, mesothelioma malignant, metastatic, epididymis																										1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Serosa, mesothelioma malignant, metastatic, epididymis																										1
Intestine small, jejunum	+	+	A	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Serosa, mesothelioma malignant, metastatic, epididymis																										1
Intestine small, ileum	+	+	A	+	+	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Serosa, mesothelioma malignant, metastatic, epididymis																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma				X																						3
Mesentery																								+		7
Mesothelioma malignant, metastatic, epididymis																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Serosa, mesothelioma malignant, metastatic, epididymis																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign			X				X			X						X	X							X		X	15
Bilateral, pheochromocytoma benign	X	X		X	X	X			X			X		X				X	X		X		X		X		19
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X												X						2
Carcinoma						X																				2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																										1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X			X	X							X		X	X			X					X	X	11
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, follicular cell, adenoma																										1
C-cell, adenoma										X																2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm (continued)

Number of Days on Study	4 5 8	4 7 0	4 8 5	5 3 1	5 3 8	5 4 3	5 5 8	5 6 1	5 6 1	5 7 7	5 7 1	5 9 2	5 9 4	6 1 0	6 1 4	6 1 8	6 2 4	6 2 6	6 3 2	6 3 3	6 3 6	6 3 8	6 4 6	6 5 0	6 7 0
Carcass ID Number	0 7 6	0 7 7	0 8 6	0 7 3	0 8 3	0 9 8	0 9 5	0 5 4	0 5 6	0 6 6	0 6 7	0 5 5	0 6 4	0 7 4	0 7 1	0 8 5	0 7 5	0 5 9	0 7 0	0 6 2	0 5 2	0 5 3	0 5 5	0 8 7	0 9 2
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X																X			X			
Carcinoma																X									
Bilateral, carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis																							X		
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis																							X		
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis																							X		
Bilateral, interstitial cell, adenoma	X	X		X	X	X	X			X	X		X	X		X	X	X	X	X	X	X	X	X	
Interstitial cell, adenoma			X					X																	X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+			+			+	+		+	+			+	+		+	+	+	+			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Mesothelioma malignant, metastatic, epididymis																							X		
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Squamous cell carcinoma																									
Subcutaneous tissue, fibroma								X			X														
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle										+							+					+	+		
Mesothelioma malignant, metastatic, epididymis																							X		
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve										+							+								
Spinal cord										+							+								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm (continued)

Number of Days on Study	6 7 9	6 8 5	6 9 1	6 9 3	6 9 4	7 0 1	7 1 0	7 1 5	7 2 3	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 5 1	0 6 1	0 8 9	0 7 9	0 9 6	0 9 4	0 6 8	0 7 2	0 7 8	0 9 0	0 5 8	0 6 0	0 6 3	0 6 5	0 6 9	0 8 0	0 8 1	0 8 2	0 8 4	0 8 7	0 9 1	0 9 3	0 9 7	0 9 9	0 1 0	Total Tissues/ Tumors
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma								X																	4
Carcinoma																						X			2
Bilateral, carcinoma												X													1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																									1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																									1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																									1
Bilateral, interstitial cell, adenoma	X	X	X	X		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	42
Interstitial cell, adenoma					X																				4
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+		+										+		+	+			+	+				22
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma															X										1
Mesothelioma malignant, metastatic, epididymis																									1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymoma benign																		X							1
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	47
Fibroadenoma				X									X												2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma				X								X													2
Squamous cell carcinoma					X																				1
Subcutaneous tissue, fibroma																									2
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle		+																							5
Mesothelioma malignant, metastatic, epididymis																									1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve		+																							3
Spinal cord		+																							3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm (continued)

Number of Days on Study	4	4	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	
	5	7	8	3	3	4	5	6	6	7	9	9	9	1	1	1	2	2	3	3	3	3	4	5	7
	8	0	5	1	8	3	8	1	1	7	1	2	4	0	4	8	4	6	2	3	6	8	6	0	0
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	7	7	8	7	8	9	9	5	5	6	6	5	6	7	7	8	7	5	7	6	5	5	5	8	9
	6	7	6	3	3	8	5	4	6	6	7	5	4	4	1	5	5	9	0	2	2	3	7	8	2
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Chordoma, metastatic, uncertain primary site																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma										X															
Renal tubule, carcinoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serosa, mesothelioma malignant, metastatic, epididymis																							X		
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X	X		X	X		X	X	X		X	X	X		X	X			X	X	X			X
Mesothelioma malignant																							X		X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm (continued)

Number of Days on Study	6 7 9	6 8 5	6 9 1	6 9 3	6 9 4	7 0 1	7 1 0	7 1 5	7 2 3	7 2 8	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 5 1	0 6 1	0 8 9	0 7 9	0 9 6	0 9 4	0 6 8	0 7 2	0 7 8	0 9 0	0 5 8	0 6 0	0 6 3	0 6 5	0 6 9	0 8 0	0 8 1	0 8 2	0 8 4	0 8 7	0 9 1	0 9 3	0 9 7	0 9 9	1 0 0	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																									X	1
Alveolar/bronchiolar carcinoma														X												1
Chordoma, metastatic, uncertain primary site							X																			1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																								+		1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma									X							X					X					4
Renal tubule, carcinoma				X																						1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Serosa, mesothelioma malignant, metastatic, epididymis																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X					X		X	X						X		X	X	X	X					27
Mesothelioma malignant																										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 25,000 ppm

Number of Days on Study	3 7 2	3 8 3	4 1 6	4 4 4	4 8 2	5 8 4	5 9 1	6 0 1	6 2 2	6 2 3	6 2 3	6 2 2	6 3 3	6 3 4	6 3 6	6 4 5	6 5 4	6 6 1	6 6 1	6 6 4	6 6 6	6 7 0	7 7 1	7 8 1
Carcass ID Number	1 4 1	1 3 4	1 3 1	1 4 7	1 4 3	1 0 2	1 4 9	1 2 1	1 1 2	1 1 3	1 3 9	1 3 5	1 3 7	1 3 2	1 4 2	1 5 0	1 3 8	1 1 4	1 2 2	2 2 4	2 2 3	2 3 3	1 1 8	1 4 0	1 1 9
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	M	A	+	A	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	A	+	A	+	A	+	+	A	A	A	A	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								
Mesentery	+												+											
Histiocytic sarcoma																								
Oral mucosa																				+				
Pharyngeal, squamous cell carcinoma																				X				
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								
Acinus, adenoma																								
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																								
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign	X								X				X	X	X			X						
Bilateral, pheochromocytoma malignant																X								
Bilateral, pheochromocytoma benign							X			X	X						X		X		X		X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																								
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X								X					X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma											X													
C-cell, carcinoma																								
Follicular cell, adenoma																								
General Body System																								
None																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 25,000 ppm (continued)

Number of Days on Study	6 8 1	6 9 2	6 9 7	6 9 8	7 0 1	7 0 4	7 1 0	7 1 3	7 2 0	7 2 2 7	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	7 2 2 9	
Carcass ID Number	1 2 0	1 1	1 2 9	1 4 5	1 1 0	1 4 8	1 0 3	1 3 0	1 0 9	1 0 0 8	1 0 1	1 0 4	1 0 5	1 0 6	1 0 7	1 1 5	1 1 6	1 1 7	1 2 5	1 2 6	1 2 7	1 2 8	1 3 6	1 4 4	1 4 6	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	A	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																X									1
Mesentery																+									3
Histiocytic sarcoma																X									1
Oral mucosa																									1
Pharyngeal, squamous cell carcinoma																									1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																X									1
Acinus, adenoma										X	X														2
Salivary glands	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant	X																								1
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign	X	X			X										X	X				X	X		X		X	15
Bilateral, pheochromocytoma malignant																									1
Bilateral, pheochromocytoma benign			X			X	X			X			X	X			X	X	X			X		X		19
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma	X																								1
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X			X		X	X	X		X		X			X			X	X		X		X	X	16
Thyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma							X								X										3
C-cell, carcinoma													X												1
Follicular cell, adenoma												X						X							2
General Body System																									
None																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 25,000 ppm (continued)

Number of Days on Study	3 7 2	3 8 3	4 1 6	4 4 4	4 8 2	5 8 4	5 9 1	6 0 1	6 2 2	6 2 3	6 2 3	6 3 2	6 3 3	6 3 4	6 3 6	6 4 5	6 5 4	6 6 1	6 6 1	6 6 4	6 6 6	6 7 0	7 7 1	7 8 1
Carcass ID Number	1 4 1	1 3 4	1 3 1	1 4 7	1 4 3	1 0 2	1 4 9	1 2 1	1 1 2	1 1 3	1 3 9	1 3 5	1 3 7	1 3 2	1 4 2	1 5 0	1 3 8	1 1 4	1 2 2	1 2 4	1 2 3	1 3 3	1 1 8	1 4 0	1 1 9
Genital System																								
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X																					
Carcinoma																								
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma	X																							
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+				+	+	+	+	+	+	+		+	+		+	+	+	+			+		
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Histiocytic sarcoma																								
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma			X																					
Histiocytic sarcoma																								
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Histiocytic sarcoma																								
Integumentary System																								
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Fibroadenoma																								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																								
Keratoacanthoma									X															
Subcutaneous tissue, fibroma																								
Subcutaneous tissue, histiocytic sarcoma																								
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+					+																		
Osteosarcoma																								
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve	+					+																		
Spinal cord	+					+																		
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								
Osteosarcoma, metastatic, skeletal muscle																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, oral mucosa																				X				
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 25,000 ppm (continued)

Number of Days on Study	6 8 1	6 9 2	6 9 7	6 9 8	7 0 1	7 0 4	7 1 0	7 1 3	7 2 0	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	1 2 0	1 1 1	1 2 9	1 4 5	1 1 0	1 4 8	1 0 3	1 3 0	1 0 9	1 0 0 8	1 0 0 1	1 0 0 4	1 0 0 5	1 0 0 6	1 0 0 7	1 1 1 5	1 1 1 6	1 1 1 7	1 2 2 5	1 2 2 6	1 2 2 7	1 2 2 8	1 3 3 6	1 4 4 4	1 4 4 6	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma					X					X											X					4
Carcinoma																				X	X					2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	46
Interstitial cell, adenoma																		X	X							3
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+				+	+	+	+					+		+			+		+	+			27
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	47
Histiocytic sarcoma																X										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Histiocytic sarcoma																X										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Histiocytic sarcoma																X										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma													X													1
Fibroadenoma																					X				X	2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma												X														1
Keratoacanthoma												X			X											3
Subcutaneous tissue, fibroma																							X			1
Subcutaneous tissue, histiocytic sarcoma																X										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle		+																					+			4
Osteosarcoma																							X			1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve		+																								3
Spinal cord		+																								3
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																X										1
Osteosarcoma, metastatic, skeletal muscle																							X			1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, oral mucosa																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 25,000 ppm (continued)

Number of Days on Study	3	3	4	4	4	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	7	8	1	4	8	8	9	0	2	2	2	3	3	3	3	4	5	6	6	6	6	7	7	7	8
	2	3	6	4	2	4	1	1	2	3	3	2	3	4	6	5	4	1	1	4	6	0	1	1	1
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	4	3	3	4	4	0	4	2	1	1	3	3	3	3	4	5	3	1	2	2	2	3	1	4	1
	1	4	1	7	3	2	9	1	2	3	9	5	7	2	2	0	8	4	2	4	3	3	8	0	9
Special Senses System																									
Ear	+																								
Zymbal's gland																									
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Renal tubule, carcinoma																									
Transitional epithelium, carcinoma																								X	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Leukemia mononuclear		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 Phenolphthalein: 25,000 ppm (continued)

Number of Days on Study	6 8 1	6 9 2	6 9 7	6 9 8	7 0 1	7 0 4	7 1 0	7 1 3	7 2 0	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	1 2 0	1 1 1	1 2 9	1 4 5	1 1 0	1 4 8	1 0 3	1 3 0	1 0 9	1 0 8	1 0 1	1 0 4	1 0 5	1 0 6	1 0 7	1 1 5	1 1 6	1 1 7	1 2 5	1 2 6	1 2 7	1 2 8	1 3 6	1 4 4	1 4 6	Total Tissues/ Tumors
Special Senses System																										
Ear																										1
Zymbal's gland					+																					1
Adenoma					X																					1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma		X																					X			2
Renal tubule, carcinoma										X																1
Transitional epithelium, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																X										1
Leukemia mononuclear	X		X			X		X	X								X	X	X			X	X			24

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 50,000 ppm

Number of Days on Study	4 8 5	5 2 4	5 2 8	5 4 1	5 4 2	5 6 3	5 7 0	5 8 5	5 9 1	5 9 4	6 1 2	6 2 2	6 2 4	6 2 6	6 3 3	6 3 6	6 4 5	6 5 2	6 5 4	6 5 9	6 6 4	6 6 4	6 6 5	6 6 8	6 7 1
Carcass ID Number	1 5 9	1 8 0	1 6 7	1 9 0	1 8 5	1 7 9	1 6 4	1 9 4	1 6 6	1 8 7	1 9 3	1 5 2	1 5 6	1 7 5	1 9 8	1 6 3	1 8 3	1 6 8	1 6 2	1 6 6	1 7 1	1 8 8	1 9 9	1 9 5	1 6 9	1 9 6
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	A	+	+	M	A	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Intestine small, jejunum	+	A	+	+	A	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	M	A	+
Intestine small, ileum	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	M	A	A
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																								X	
Hepatocellular adenoma, multiple																									
Mesentery			+														+								
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	A	+
Mesothelioma malignant, metastatic, epididymis																X									
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Tooth															+										
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign			X		X				X	X		X		X	X	X				X	X		X	X	
Bilateral, pheochromocytoma benign				X				X			X		X					X	X						X
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Adenoma											X														
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X			X							X									X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
C-cell, adenoma							X								X										X
C-cell, carcinoma																									
Follicular cell, adenoma																									
General Body System																									
None																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 50,000 ppm (continued)

Number of Days on Study	6 7 3	6 7 3	6 8 5	6 9 3	6 9 6	7 0 0	7 1 0	7 1 1	7 1 7	7 1 7	7 1 8	7 2 0	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	1 5 8	1 7 3	1 5 1	1 9 2	1 6 5	1 8 8	1 7 6	1 5 4	1 9 1	1 9 9	1 8 4	1 7 1	1 5 3	1 5 5	1 5 7	1 6 0	1 7 0	1 7 2	1 7 4	1 7 7	1 8 1	1 8 2	1 8 6	1 9 7	2 0 0	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	A	+	+	+	A	+	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Intestine small, ileum	A	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																										1
Hepatocellular adenoma, multiple [1]							X																			1
Mesentery				+																						3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Mesothelioma malignant, metastatic, epididymis																										1
Acinus, adenoma																							X			1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant									X										X							2
Pheochromocytoma benign		X							X					X	X			X			X			X		19
Bilateral, pheochromocytoma benign					X	X		X		X			X				X					X	X			15
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																										1
Carcinoma						X	X																			2
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma							X				X		X	X							X	X			X	11
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma		X																								4
C-cell, carcinoma						X					X															2
Follicular cell, adenoma			X			X														X						3
General Body System																										
None																										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 50,000 ppm (continued)

Number of Days on Study	485	524	528	541	542	563	570	585	591	594	612	622	624	626	633	636	645	652	654	659	664	664	665	668	671
Carcass ID Number	159	180	167	190	185	179	164	194	186	187	193	152	156	175	198	163	183	168	162	161	178	189	195	169	196
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X																	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, epididymis																X	X								
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Interstitial cell, adenoma								X															X		X
Hematopoietic System																									
Bone marrow	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node			+	+			+	+	+			+	+	+		+	+	+	+					+	
Mediastinal, carcinoma, metastatic, kidney																									
Renal, carcinoma, metastatic, kidney																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	A	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma			X																						
Pinna, schwannoma malignant							X																		
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, schwannoma malignant									X																
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranium, osteosarcoma															X										
Skeletal muscle														+											
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic, kidney																									
Osteosarcoma, metastatic, bone															X										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye															+										
Zymbal's gland											+														
Carcinoma											X														

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 50,000 ppm (continued)

Number of Days on Study	6 7 3	6 7 3	6 8 5	6 9 3	6 9 6	7 0 0	7 1 0	7 1 1	7 1 7	7 1 7	7 1 8	7 2 0	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	1 5 8	1 7 3	1 5 1	1 9 2	1 6 5	1 8 8	1 7 6	1 5 4	1 9 1	1 9 9	1 8 4	1 7 1	1 5 3	1 5 5	1 5 7	1 6 0	1 7 0	1 7 2	1 7 4	1 7 7	1 8 1	1 8 2	1 8 6	1 9 7	2 0 0	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma											X								X		X					4
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, epididymis																										2
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		45
Interstitial cell, adenoma																									X	4
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+				+		+											+	+	+	+					20
Mediastinal, carcinoma, metastatic, kidney							X																			1
Renal, carcinoma, metastatic, kidney							X																			1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Fibroadenoma																					X					1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																										1
Pinna, schwannoma malignant																					X					2
Subcutaneous tissue, fibroma	X	X																								2
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cranium, osteosarcoma																										1
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																							X			1
Carcinoma, metastatic, kidney							X																			1
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																									+	2
Zymbal's gland																				+						2
Carcinoma																				X						2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 50,000 ppm (continued)

Number of Days on Study	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	8	2	2	4	4	6	7	8	9	9	1	2	2	2	3	3	4	5	5	5	6	6	6	7
	5	4	8	1	2	3	0	5	1	4	2	2	4	6	3	6	5	2	4	9	4	4	5	8	1
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	8	6	9	8	7	6	9	6	8	9	5	5	7	9	6	8	6	6	6	7	8	9	6	9
	9	0	7	0	5	9	4	4	6	7	3	2	6	5	8	3	3	8	2	1	8	9	5	9	6
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma					X									X					X					X	
Renal tubule, carcinoma																		X							
Renal tubule, oncocytoma benign																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	X	X		X	X	X	X		X	X		X	X	X		X			X	X			
Mesothelioma malignant																X	X								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Phenolphthalein: 50,000 ppm (continued)

Number of Days on Study	6 7 3	6 7 3	6 8 5	6 9 3	6 9 6	7 0 0	7 1 0	7 1 1	7 1 7	7 1 7	7 1 8	7 2 0	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	1 5 8	1 7 3	1 5 1	1 9 2	1 6 5	1 8 8	1 7 6	1 5 4	1 9 1	1 9 9	1 8 4	1 7 1	1 5 3	1 5 5	1 5 7	1 6 0	1 7 0	1 7 2	1 7 4	1 7 7	1 8 1	1 8 2	1 8 6	1 9 7	2 0 0	Total Tissues/ Tumors
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma							X											X							6
Renal tubule, carcinoma							X																		2
Renal tubule, oncocytoma benign											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	X	29
Mesothelioma malignant																									2

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

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	17/50 (34%)	34/50 (68%)	34/50 (68%)	34/50 (68%)
Adjusted rate[b]	62.1%	88.6%	93.7%	87.0%
Terminal rate[c]	11/21 (52%)	11/15 (73%)	13/15 (87%)	9/13 (69%)
First incidence (days)	608	485	372	528
Life table test[d]	P=0.003	P<0.001	P<0.001	P<0.001
Logistic regression test[d]	P=0.006	P<0.001	P<0.001	P=0.001
Cochran-Armitage test[d]	P=0.003			
Fisher exact test[d]		P<0.001	P<0.001	P<0.001
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	18/50 (36%)	35/50 (70%)	35/50 (70%)	35/50 (70%)
Adjusted rate	62.9%	88.9%	93.9%	90.3%
Terminal rate	11/21 (52%)	11/15 (73%)	13/15 (87%)	10/13 (77%)
First incidence (days)	330	485	372	528
Life table test	P=0.003	P<0.001	P<0.001	P<0.001
Logistic regression test	P=0.005	P<0.001	P<0.001	P=0.001
Cochran-Armitage test	P=0.003			
Fisher exact test		P<0.001	P<0.001	P<0.001
Kidney (Renal Tubule): Adenoma (Single Sections)				
Overall rate	0/50 (0%)	4/50 (8%)	2/50 (4%)	6/50 (12%)
Adjusted rate	0.0%	20.4%	10.6%	22.4%
Terminal rate	0/21 (0%)	2/15 (13%)	1/15 (7%)	1/13 (8%)
First incidence (days)	—[e]	577	692	542
Life table test	P=0.015	P=0.040	P=0.195	P=0.017
Logistic regression test	P=0.026	P=0.060	P=0.233	P=0.018
Cochran-Armitage test	P=0.023			
Fisher exact test		P=0.059	P=0.247	P=0.013
Kidney (Renal Tubule): Adenoma (Step Sections)				
Overall rate	1/50 (2%)	7/50 (14%)	15/50 (30%)	11/50 (22%)
Adjusted rate	4.8%	36.4%	64.9%	51.1%
Terminal rate	1/21 (5%)	4/15 (27%)	8/15 (53%)	5/13 (38%)
First incidence (days)	729 (T)	691	664	542
Life table test	P<0.001	P=0.012	P<0.001	P<0.001
Logistic regression test	P=0.003	P=0.017	P<0.001	P=0.003
Cochran-Armitage test	P=0.005			
Fisher exact test		P=0.030	P<0.001	P=0.002
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	1/50 (2%)	10/50 (20%)	15/50 (30%)	15/50 (30%)
Adjusted rate	4.8%	46.9%	64.9%	61.8%
Terminal rate	1/21 (5%)	5/15 (33%)	8/15 (53%)	6/13 (46%)
First incidence (days)	729 (T)	577	664	542
Life table test	P<0.001	P=0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.003	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.004	P<0.001	P<0.001

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Kidney (Renal Tubule): Adenoma or Carcinoma (Single Sections)				
Overall rate	0/50 (0%)	5/50 (10%)	3/50 (6%)	7/50 (14%)
Adjusted rate	0.0%	24.0%	16.1%	24.7%
Terminal rate	0/21 (0%)	2/15 (13%)	1/15 (7%)	1/13 (8%)
First incidence (days)	—	577	692	542
Life table test	P=0.011	P=0.020	P=0.084	P=0.011
Logistic regression test	P=0.020	P=0.031	P=0.106	P=0.009
Cochran-Armitage test	P=0.017			
Fisher exact test		P=0.028	P=0.121	P=0.006
Kidney (Renal Tubule): Adenoma or Carcinoma (Step Sections)				
Overall rate	1/50 (2%)	7/50 (14%)	15/50 (30%)	11/50 (22%)
Adjusted rate	4.8%	36.4%	64.9%	51.1%
Terminal rate	1/21 (5%)	4/15 (27%)	8/15 (53%)	5/13 (38%)
First incidence (days)	729 (T)	691	664	542
Life table test	P<0.001	P=0.012	P<0.001	P<0.001
Logistic regression test	P=0.003	P=0.017	P<0.001	P=0.003
Cochran-Armitage test	P=0.005			
Fisher exact test		P=0.030	P<0.001	P=0.002
Kidney (Renal Tubule): Adenoma or Carcinoma (Single and Step Sections)				
Overall rate	1/50 (2%)	10/50 (20%)	16/50 (32%)	16/50 (32%)
Adjusted rate	4.8%	46.9%	67.1%	62.9%
Terminal rate	1/21 (5%)	5/15 (33%)	8/15 (53%)	6/13 (46%)
First incidence (days)	729 (T)	577	664	542
Life table test	P<0.001	P=0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.003	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.004	P<0.001	P<0.001
Liver: Hepatocellular Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	4.5%	9.4%	0.0%	8.8%
Terminal rate	0/21 (0%)	0/15 (0%)	0/15 (0%)	0/13 (0%)
First incidence (days)	727	577	—	668
Life table test	P=0.532	P=0.285	P=0.564N	P=0.409
Logistic regression test	P=0.571	P=0.307	P=0.554N	P=0.495
Cochran-Armitage test	P=0.562			
Fisher exact test		P=0.309	P=0.500N	P=0.500
Mammary Gland: Fibroadenoma				
Overall rate	6/50 (12%)	2/50 (4%)	2/50 (4%)	1/50 (2%)
Adjusted rate	22.8%	10.9%	13.3%	7.7%
Terminal rate	3/21 (14%)	1/15 (7%)	2/15 (13%)	1/13 (8%)
First incidence (days)	534	693	729 (T)	729 (T)
Life table test	P=0.092N	P=0.231N	P=0.235N	P=0.131N
Logistic regression test	P=0.041N	P=0.138N	P=0.120N	P=0.054N
Cochran-Armitage test	P=0.043N			
Fisher exact test		P=0.134N	P=0.134N	P=0.056N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	7/50 (14%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	26.0%	10.9%	20.0%	7.7%
Terminal rate	3/21 (14%)	1/15 (7%)	3/15 (20%)	1/13 (8%)
First incidence (days)	534	693	729 (T)	729 (T)
Life table test	P=0.075N	P=0.162N	P=0.295N	P=0.088N
Logistic regression test	P=0.030N	P=0.084N	P=0.142N	P=0.030N
Cochran-Armitage test	P=0.030N			
Fisher exact test		P=0.080N	P=0.159N	P=0.030N
Pancreatic Islets: Adenoma				
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)	1/48 (2%)
Adjusted rate	13.6%	11.9%	0.0%	2.5%
Terminal rate	2/21 (10%)	1/15 (7%)	0/15 (0%)	0/13 (0%)
First incidence (days)	727	715	—	612
Life table test	P=0.242N	P=0.644N	P=0.186N	P=0.425N
Logistic regression test	P=0.169N	P=0.612N	P=0.175N	P=0.311N
Cochran-Armitage test	P=0.171N			
Fisher exact test		P=0.500N	P=0.121N	P=0.324N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	1/50 (2%)	3/48 (6%)
Adjusted rate	18.2%	19.1%	3.8%	12.2%
Terminal rate	3/21 (14%)	1/15 (7%)	0/15 (0%)	0/13 (0%)
First incidence (days)	727	638	681	612
Life table test	P=0.466N	P=0.483	P=0.271N	P=0.635
Logistic regression test	P=0.344N	P=0.593	P=0.199N	P=0.525N
Cochran-Armitage test	P=0.361N			
Fisher exact test		P=0.643N	P=0.181N	P=0.523N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	13/50 (26%)	11/50 (22%)	16/50 (32%)	11/50 (22%)
Adjusted rate	42.9%	50.8%	67.3%	51.3%
Terminal rate	6/21 (29%)	6/15 (40%)	8/15 (53%)	5/13 (38%)
First incidence (days)	407	610	623	542
Life table test	P=0.362	P=0.555	P=0.169	P=0.528
Logistic regression test	P=0.390N	P=0.391N	P=0.388	P=0.367N
Cochran-Armitage test	P=0.449N			
Fisher exact test		P=0.408N	P=0.330	P=0.408N
Preputial Gland: Adenoma				
Overall rate	5/50 (10%)	4/49 (8%)	4/50 (8%)	4/49 (8%)
Adjusted rate	21.9%	13.5%	18.4%	22.9%
Terminal rate	4/21 (19%)	0/15 (0%)	1/15 (7%)	2/13 (15%)
First incidence (days)	671	485	416	585
Life table test	P=0.543	P=0.613N	P=0.625	P=0.561
Logistic regression test	P=0.439N	P=0.502N	P=0.472N	P=0.539N
Cochran-Armitage test	P=0.458N			
Fisher exact test		P=0.513N	P=0.500N	P=0.513N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Preputial Gland: Carcinoma				
Overall rate	0/50 (0%)	3/49 (6%)	2/50 (4%)	0/49 (0%)
Adjusted rate	0.0%	15.8%	13.3%	0.0%
Terminal rate	0/21 (0%)	2/15 (13%)	2/15 (13%)	0/13 (0%)
First incidence (days)	—	618	729 (T)	—
Life table test	P=0.498N	P=0.092	P=0.166	—
Logistic regression test	P=0.419N	P=0.114	P=0.166	—
Cochran-Armitage test	P=0.405N			
Fisher exact test		P=0.117	P=0.247	—
Preputial Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	7/49 (14%)	5/50 (10%)	4/49 (8%)
Adjusted rate	21.9%	27.2%	24.2%	22.9%
Terminal rate	4/21 (19%)	2/15 (13%)	2/15 (13%)	2/13 (15%)
First incidence (days)	671	485	416	585
Life table test	P=0.491N	P=0.263	P=0.466	P=0.561
Logistic regression test	P=0.316N	P=0.375	P=0.602N	P=0.539N
Cochran-Armitage test	P=0.339N			
Fisher exact test		P=0.365	P=0.630N	P=0.513N
Skin: Keratoacanthoma				
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	17.5%	10.9%	15.4%	2.1%
Terminal rate	3/21 (14%)	1/15 (7%)	2/15 (13%)	0/13 (0%)
First incidence (days)	685	693	622	528
Life table test	P=0.245N	P=0.468N	P=0.614N	P=0.276N
Logistic regression test	P=0.153N	P=0.391N	P=0.493N	P=0.168N
Cochran-Armitage test	P=0.164N			
Fisher exact test		P=0.339N	P=0.500N	P=0.181N
Skin: Keratoacanthoma or Squamous Cell Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	17.5%	15.2%	15.4%	2.1%
Terminal rate	3/21 (14%)	1/15 (7%)	2/15 (13%)	0/13 (0%)
First incidence (days)	685	693	622	528
Life table test	P=0.212N	P=0.640N	P=0.614N	P=0.276N
Logistic regression test	P=0.125N	P=0.556N	P=0.493N	P=0.168N
Cochran-Armitage test	P=0.136N			
Fisher exact test		P=0.500N	P=0.500N	P=0.181N
Skin: Keratoacanthoma, Basal Cell Adenoma, or Squamous Cell Carcinoma				
Overall rate	5/50 (10%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	22.0%	15.2%	15.4%	2.1%
Terminal rate	4/21 (19%)	1/15 (7%)	2/15 (13%)	0/13 (0%)
First incidence (days)	685	693	622	528
Life table test	P=0.141N	P=0.512N	P=0.484N	P=0.190N
Logistic regression test	P=0.073N	P=0.416N	P=0.353N	P=0.095N
Cochran-Armitage test	P=0.080N			
Fisher exact test		P=0.357N	P=0.357N	P=0.102N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	2/50 (4%)
Adjusted rate	10.8%	4.8%	6.7%	8.0%
Terminal rate	1/21 (5%)	0/15 (0%)	1/15 (7%)	0/13 (0%)
First incidence (days)	534	561	729 (T)	673
Life table test	P=0.448N	P=0.526N	P=0.385N	P=0.568N
Logistic regression test	P=0.407N	P=0.518N	P=0.292N	P=0.488N
Cochran-Armitage test	P=0.404N			
Fisher exact test		P=0.500N	P=0.309N	P=0.500N
Skin (Pinna and Subcutaneous Tissue): Malignant Schwannoma				
Overall rate	2/50 (4%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	7.6%	0.0%	0.0%	11.9%
Terminal rate	1/21 (5%)	0/15 (0%)	0/15 (0%)	1/13 (8%)
First incidence (days)	608	—	—	570
Life table test	P=0.220	P=0.266N	P=0.251N	P=0.457
Logistic regression test	P=0.234	P=0.235N	P=0.231N	P=0.493
Cochran-Armitage test	P=0.241			
Fisher exact test		P=0.247N	P=0.247N	P=0.500
Testes: Adenoma				
Overall rate	46/50 (92%)	46/50 (92%)	49/50 (98%)	49/50 (98%)
Adjusted rate	100.0%	97.8%	100.0%	100.0%
Terminal rate	21/21 (100%)	14/15 (93%)	15/15 (100%)	13/13 (100%)
First incidence (days)	486	458	372	485
Life table test	P=0.112	P=0.233	P=0.180	P=0.103
Logistic regression test	P=0.223	P=0.365N	P=0.225	P=0.680
Cochran-Armitage test	P=0.076			
Fisher exact test		P=0.643N	P=0.181	P=0.181
Thyroid Gland (C-cell): Adenoma				
Overall rate	9/50 (18%)	2/50 (4%)	3/49 (6%)	4/49 (8%)
Adjusted rate	35.3%	8.2%	13.7%	12.3%
Terminal rate	6/21 (29%)	0/15 (0%)	1/15 (7%)	0/13 (0%)
First incidence (days)	571	531	623	570
Life table test	P=0.216N	P=0.061N	P=0.111N	P=0.214N
Logistic regression test	P=0.137N	P=0.026N	P=0.055N	P=0.108N
Cochran-Armitage test	P=0.152N			
Fisher exact test		P=0.026N	P=0.065N	P=0.125N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	9/50 (18%)	2/50 (4%)	4/49 (8%)	6/49 (12%)
Adjusted rate	35.3%	8.2%	19.9%	22.2%
Terminal rate	6/21 (29%)	0/15 (0%)	2/15 (13%)	0/13 (0%)
First incidence (days)	571	531	623	570
Life table test	P=0.532N	P=0.061N	P=0.200N	P=0.459N
Logistic regression test	P=0.386N	P=0.026N	P=0.106N	P=0.271N
Cochran-Armitage test	P=0.417N			
Fisher exact test		P=0.026N	P=0.125N	P=0.303N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/50 (0%)	1/50 (2%)	2/49 (4%)	3/49 (6%)
Adjusted rate	0.0%	3.6%	13.3%	16.1%
Terminal rate	0/21 (0%)	0/15 (0%)	2/15 (13%)	1/13 (8%)
First incidence (days)	—	646	729 (T)	685
Life table test	P=0.039	P=0.493	P=0.166	P=0.076
Logistic regression test	P=0.057	P=0.500	P=0.166	P=0.110
Cochran-Armitage test	P=0.059			
Fisher exact test		P=0.500	P=0.242	P=0.117
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	2/49 (4%)	3/49 (6%)
Adjusted rate	2.4%	3.6%	13.3%	16.1%
Terminal rate	0/21 (0%)	0/15 (0%)	2/15 (13%)	1/13 (8%)
First incidence (days)	529	646	729 (T)	685
Life table test	P=0.121	P=0.752N	P=0.430	P=0.241
Logistic regression test	P=0.168	P=0.735	P=0.500	P=0.305
Cochran-Armitage test	P=0.158			
Fisher exact test		P=0.753N	P=0.492	P=0.301
All Organs: Mononuclear Cell Leukemia				
Overall rate	31/50 (62%)	27/50 (54%)	24/50 (48%)	29/50 (58%)
Adjusted rate	76.0%	67.6%	65.0%	87.4%
Terminal rate	12/21 (57%)	5/15 (33%)	5/15 (33%)	10/13 (77%)
First incidence (days)	330	458	383	524
Life table test	P=0.448	P=0.503N	P=0.284N	P=0.412
Logistic regression test	P=0.457N	P=0.291N	P=0.116N	P=0.421N
Cochran-Armitage test	P=0.416N			
Fisher exact test		P=0.272N	P=0.114N	P=0.419N
All Organs: Benign Neoplasms				
Overall rate	47/50 (94%)	50/50 (100%)	49/50 (98%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	21/21 (100%)	15/15 (100%)	15/15 (100%)	13/13 (100%)
First incidence (days)	407	458	372	485
Life table test	P=0.148	P=0.136	P=0.219	P=0.107
Logistic regression test	P=0.354	P=0.456	P=0.480	P=0.602
Cochran-Armitage test	P=0.089			
Fisher exact test		P=0.121	P=0.309	P=0.121
All Organs: Malignant Neoplasms				
Overall rate	35/50 (70%)	36/50 (72%)	31/50 (62%)	33/50 (66%)
Adjusted rate	80.2%	84.2%	81.6%	89.6%
Terminal rate	13/21 (62%)	9/15 (60%)	9/15 (60%)	10/13 (77%)
First incidence (days)	330	458	383	524
Life table test	P=0.467	P=0.297	P=0.491N	P=0.399
Logistic regression test	P=0.297N	P=0.486	P=0.253N	P=0.420N
Cochran-Armitage test	P=0.294N			
Fisher exact test		P=0.500	P=0.263N	P=0.415N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	21/21 (100%)	15/15 (100%)	15/15 (100%)	13/13 (100%)
First incidence (days)	330	458	372	485
Life table test	P=0.201	P=0.206	P=0.268	P=0.167
Logistic regression test	—[f]	—	—	—
Cochran-Armitage test	P=0.309			
Fisher exact test		P=0.500	P=0.500	P=0.500

(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, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, 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 Adrenal Medulla Pheochromocytoma in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Benign	Malignant	Benign or Malignant
Historical Incidence at EG&G Mason Research Institute/TSI Mason Laboratories, Inc.			
1-Amino-2,4-dibromoanthraquinone	12/50	1/50	13/50
Acetaminophen	16/44	1/44	17/44
HC Yellow 4	19/50	2/50	19/50
Methylphenidate Hydrochloride	17/49	1/49	18/49
Pentaerythritol Tetranitrate	19/49	0/49	19/49
Quercetin	12/50	1/50	13/50
Turmeric Oleoresin	14/47	0/47	14/47
Overall Historical Incidence			
Total	396/1,283 (30.9%)	39/1,283 (3.0%)	421/1,283 (32.8%)
Standard deviation	12.1%	3.1%	11.2%
Range	10%-63%	0%-12%	14%-63%

[a] Data as of 12 May 1995; includes data for complex pheochromocytoma

TABLE A4b
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 EG&G Mason Research Institute/TSI Mason Laboratories, Inc.			
1-Amino-2,4-dibromoanthraquinone	2/50	0/50	2/50
Acetaminophen	3/50	0/50	3/50
HC Yellow 4	1/50	0/50	1/50
Methylphenidate Hydrochloride	0/49	0/49	0/49
Pentaerythritol Tetranitrate	0/49	0/49	0/49
Quercetin	0/50	0/50	0/50
Turmeric Oleoresin	0/50	0/50	0/50
Overall Historical Incidence			
Total	9/1,301 (0.7%)	3/1,301 (0.2%)	12/1,301 (0.9%)
Standard deviation	1.5%	0.7%	1.5%
Range	0%-6%	0%-2%	0%-6%

[a] Data as of 12 May 1995

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

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	15	13	16
Natural deaths	14	20	22	21
Survivors				
Died last week of the study			1	
Terminal sacrifice	21	15	14	13
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(48)	(49)	(49)	(48)
Parasite metazoan	1 (2%)	2 (4%)	4 (8%)	3 (6%)
Muscularis, necrosis, focal				1 (2%)
Intestine large, rectum	(48)	(49)	(50)	(50)
Necrosis		1 (2%)		
Parasite metazoan	1 (2%)	2 (4%)		
Intestine large, cecum	(44)	(41)	(44)	(40)
Edema		3 (7%)	1 (2%)	1 (3%)
Erosion, focal				1 (3%)
Inflammation, acute			1 (2%)	
Inflammation, chronic			1 (2%)	
Necrosis			1 (2%)	1 (3%)
Artery, inflammation, chronic		2 (5%)		1 (3%)
Epithelium, hyperplasia				1 (3%)
Intestine small, duodenum	(47)	(50)	(49)	(47)
Artery, inflammation, chronic			1 (2%)	
Muscularis, necrosis, focal				1 (2%)
Intestine small, jejunum	(47)	(41)	(47)	(42)
Inflammation, chronic active	1 (2%)			
Intestine small, ileum	(44)	(40)	(40)	(41)
Inflammation, chronic		1 (3%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis, focal	7 (14%)	1 (2%)	1 (2%)	1 (2%)
Basophilic focus	17 (34%)	18 (36%)	9 (18%)	21 (42%)
Clear cell focus	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Degeneration, cystic, focal	4 (8%)	1 (2%)	6 (12%)	5 (10%)
Eosinophilic focus	8 (16%)	10 (20%)	7 (14%)	14 (28%)
Hematopoietic cell proliferation	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Hepatodiaphragmatic nodule	4 (8%)		1 (2%)	2 (4%)
Mixed cell focus	4 (8%)	8 (16%)	8 (16%)	
Necrosis	1 (2%)		5 (10%)	3 (6%)
Bile duct, hyperplasia	10 (20%)	1 (2%)	2 (4%)	4 (8%)
Centrilobular, necrosis	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Hepatocyte, vacuolization cytoplasmic	3 (6%)	12 (24%)	13 (26%)	10 (20%)
Vein, thrombosis	2 (4%)	1 (2%)		

[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 Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Alimentary System (continued)				
Mesentery	(20)	(7)	(3)	(3)
Metaplasia, osseous				1 (33%)
Polyarteritis			1 (33%)	
Artery, inflammation, chronic			1 (33%)	
Fat, necrosis	19 (95%)	4 (57%)	1 (33%)	2 (67%)
Pancreas	(50)	(50)	(50)	(47)
Infiltration cellular, focal, lymphocyte	1 (2%)			
Necrosis, diffuse			1 (2%)	
Pigmentation, focal		1 (2%)		
Acinus, atrophy, diffuse	1 (2%)	1 (2%)	1 (2%)	
Acinus, atrophy, focal	22 (44%)	18 (36%)	25 (50%)	31 (66%)
Acinus, hyperplasia, focal	1 (2%)	5 (10%)	4 (8%)	2 (4%)
Artery, inflammation, chronic		3 (6%)		
Salivary glands	(50)	(49)	(49)	(49)
Atrophy	1 (2%)			
Duct, sublingual gland, hyperplasia, focal	1 (2%)		1 (2%)	
Parotid gland, atrophy				1 (2%)
Parotid gland, atrophy, focal			1 (2%)	
Parotid gland, inflammation, chronic active				1 (2%)
Sublingual gland, atrophy		2 (4%)		
Sublingual gland, inflammation, chronic	1 (2%)			
Submandibular gland, atrophy				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(49)
Edema	1 (2%)	3 (6%)	1 (2%)	4 (8%)
Erosion, focal			1 (2%)	2 (4%)
Inflammation, acute			1 (2%)	
Inflammation, chronic			5 (10%)	2 (4%)
Ulcer, focal	2 (4%)	2 (4%)	4 (8%)	3 (6%)
Epithelium, hyperplasia	4 (8%)	2 (4%)	12 (24%)	9 (18%)
Muscularis, mineralization		2 (4%)	2 (4%)	
Stomach, glandular	(50)	(50)	(50)	(49)
Degeneration		11 (22%)	5 (10%)	4 (8%)
Edema			1 (2%)	
Erosion, focal	8 (16%)	6 (12%)	4 (8%)	6 (12%)
Inflammation, acute	1 (2%)			1 (2%)
Mineralization		11 (22%)	5 (10%)	5 (10%)
Mineralization, focal				1 (2%)
Ulcer, focal		1 (2%)	1 (2%)	
Glands, cyst, focal		1 (2%)	2 (4%)	3 (6%)
Glands, inflammation, acute, focal			1 (2%)	
Muscularis, mineralization			2 (4%)	
Tooth				(1)
Necrosis				1 (100%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Cardiovascular System				
Blood vessel	(50)	(49)	(50)	(50)
Aorta, media, mineralization		9 (18%)	3 (6%)	4 (8%)
Heart	(50)	(49)	(50)	(50)
Cardiomyopathy	39 (78%)	36 (73%)	41 (82%)	38 (76%)
Fibrosis				1 (2%)
Artery, mineralization		1 (2%)	2 (4%)	
Atrium, thrombosis	3 (6%)	2 (4%)	2 (4%)	
Atrium, epicardium, hyperplasia, focal	1 (2%)			
Atrium, epicardium, pigmentation	1 (2%)			
Myocardium, inflammation, acute			2 (4%)	
Myocardium, mineralization			2 (4%)	1 (2%)
Valve, inflammation, acute			1 (2%)	
Valve, inflammation, chronic active			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation		1 (2%)		1 (2%)
Hyperplasia, focal	4 (8%)	1 (2%)	1 (2%)	6 (12%)
Hypertrophy, focal	2 (4%)			
Inflammation				1 (2%)
Necrosis			2 (4%)	1 (2%)
Vacuolization cytoplasmic, focal	6 (12%)	11 (22%)	4 (8%)	7 (14%)
Bilateral, hematopoietic cell proliferation				1 (2%)
Bilateral, necrosis		4 (8%)	3 (6%)	1 (2%)
Bilateral, vacuolization cytoplasmic, focal		2 (4%)	2 (4%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia, focal	12 (24%)	21 (42%)	16 (32%)	19 (38%)
Infiltration cellular, lymphocyte	1 (2%)			
Bilateral, hyperplasia, focal	1 (2%)	1 (2%)	2 (4%)	4 (8%)
Islets, pancreatic	(50)	(50)	(50)	(48)
Hyperplasia, focal	2 (4%)			2 (4%)
Parathyroid gland	(41)	(48)	(49)	(46)
Hyperplasia, diffuse		6 (13%)	3 (6%)	8 (17%)
Hyperplasia, focal	1 (2%)			
Bilateral, hyperplasia				1 (2%)
Bilateral, hyperplasia, diffuse		10 (21%)	11 (22%)	6 (13%)
Pituitary gland	(50)	(50)	(50)	(50)
Hyperplasia, focal				1 (2%)
Pars distalis, angiectasis, focal	1 (2%)		1 (2%)	
Pars distalis, cyst	1 (2%)	4 (8%)	5 (10%)	5 (10%)
Pars distalis, hemorrhage, focal				1 (2%)
Pars distalis, hyperplasia, focal	9 (18%)	6 (12%)	4 (8%)	11 (22%)
Pars distalis, hypertrophy, focal	1 (2%)			
Pars nervosa, hyperplasia	1 (2%)	1 (2%)		
Rathke's cleft, pigmentation	1 (2%)			
Thyroid gland	(50)	(50)	(49)	(49)
Necrosis		1 (2%)		
Ultimobranchial cyst			1 (2%)	
C-cell, hyperplasia, focal	13 (26%)	3 (6%)	9 (18%)	4 (8%)
Follicle, cyst	3 (6%)	4 (8%)	7 (14%)	6 (12%)
Follicular cell, hyperplasia				1 (2%)
Follicular cell, hyperplasia, focal		1 (2%)		

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
General Body System				
Tissue NOS	(1)			
Mediastinum, hemorrhage	1 (100%)			
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm, focal				1 (2%)
Inflammation, chronic		2 (4%)	1 (2%)	
Polyarteritis			1 (2%)	
Preputial gland	(50)	(49)	(50)	(49)
Atrophy				1 (2%)
Cyst		1 (2%)		
Hyperplasia, focal	3 (6%)	2 (4%)	2 (4%)	
Inflammation, acute		1 (2%)		
Inflammation, chronic	14 (28%)	14 (29%)	8 (16%)	13 (27%)
Inflammation, chronic active	1 (2%)	2 (4%)	4 (8%)	4 (8%)
Bilateral, inflammation, chronic	21 (42%)	21 (43%)	25 (50%)	20 (41%)
Bilateral, inflammation, chronic active	6 (12%)	8 (16%)	9 (18%)	4 (8%)
Duct, hyperplasia			1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Atrophy	14 (28%)	8 (16%)	9 (18%)	13 (26%)
Inflammation, acute	6 (12%)	5 (10%)	4 (8%)	10 (20%)
Inflammation, chronic	1 (2%)	2 (4%)	1 (2%)	4 (8%)
Inflammation, chronic active	3 (6%)	7 (14%)	8 (16%)	5 (10%)
Epithelium, hyperplasia, focal	1 (2%)	2 (4%)	5 (10%)	3 (6%)
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy	33 (66%)	44 (88%)	45 (90%)	47 (94%)
Inflammation, acute	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Mineralization, focal	1 (2%)			
Bilateral, artery, inflammation, chronic		1 (2%)		
Bilateral, interstitial cell, hyperplasia, focal	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Bilateral, germinal epithelium, atrophy	4 (8%)	3 (6%)		2 (4%)
Germinal epithelium, atrophy	2 (4%)		1 (2%)	2 (4%)
Interstitial cell, hyperplasia, focal	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Hematopoietic System				
Bone marrow	(48)	(50)	(50)	(49)
Atrophy	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Fibrosis				1 (2%)
Hemorrhage				2 (4%)
Hyperplasia	15 (31%)	18 (36%)	21 (42%)	26 (53%)
Myelofibrosis	1 (2%)			
Necrosis	1 (2%)		2 (4%)	1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Hematopoietic System (continued)				
Lymph node	(17)	(22)	(27)	(20)
Infiltration cellular, histiocyte				1 (5%)
Iliac, hyperplasia, lymphoid			1 (4%)	1 (5%)
Iliac, infiltration cellular, histiocyte				1 (5%)
Inguinal, hyperplasia, lymphoid			2 (7%)	
Mediastinal, hematopoietic cell proliferation				1 (5%)
Mediastinal, hemorrhage	1 (6%)	1 (5%)	3 (11%)	1 (5%)
Mediastinal, hyperplasia, lymphoid		1 (5%)	4 (15%)	1 (5%)
Mediastinal, infiltration cellular, histiocyte			2 (7%)	1 (5%)
Mediastinal, pigmentation	1 (6%)	1 (5%)	1 (4%)	2 (10%)
Pancreatic, fibrosis				1 (5%)
Pancreatic, hematopoietic cell proliferation				1 (5%)
Pancreatic, hemorrhage	1 (6%)		1 (4%)	
Renal, congestion				1 (5%)
Renal, hemorrhage	2 (12%)	3 (14%)	5 (19%)	2 (10%)
Renal, hyperplasia, lymphoid		2 (9%)	3 (11%)	2 (10%)
Renal, infiltration cellular, histiocyte		1 (5%)	1 (4%)	2 (10%)
Renal, pigmentation		1 (5%)	1 (4%)	2 (10%)
Lymph node, mandibular	(50)	(49)	(49)	(49)
Degeneration, cystic	9 (18%)	6 (12%)	2 (4%)	5 (10%)
Hemorrhage	1 (2%)			
Hyperplasia, lymphoid	7 (14%)	6 (12%)	12 (24%)	7 (14%)
Infiltration cellular, histiocyte	2 (4%)		2 (4%)	4 (8%)
Inflammation, chronic		1 (2%)	1 (2%)	
Necrosis		1 (2%)		
Pigmentation	1 (2%)			1 (2%)
Lymph node, mesenteric	(50)	(50)	(47)	(48)
Atrophy		1 (2%)	1 (2%)	
Degeneration, cystic				1 (2%)
Degeneration, cystic, focal				1 (2%)
Ectasia				1 (2%)
Hemorrhage	4 (8%)	2 (4%)	2 (4%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)	2 (4%)	
Infiltration cellular, histiocyte	33 (66%)	18 (36%)	22 (47%)	23 (48%)
Inflammation, chronic			1 (2%)	
Spleen	(50)	(50)	(50)	(49)
Congestion	1 (2%)		6 (12%)	2 (4%)
Fibrosis	3 (6%)	8 (16%)	6 (12%)	4 (8%)
Hematopoietic cell proliferation	6 (12%)	5 (10%)	5 (10%)	6 (12%)
Inflammation, acute			1 (2%)	
Inflammation, chronic active		1 (2%)		
Pigmentation		1 (2%)	4 (8%)	2 (4%)
Capsule, degeneration, cystic	1 (2%)			
Lymphoid follicle, atrophy	2 (4%)	8 (16%)	9 (18%)	12 (24%)
Thymus	(50)	(49)	(47)	(47)
Angiectasis, focal		1 (2%)		
Cyst		1 (2%)		
Hemorrhage	3 (6%)			
Necrosis, diffuse			1 (2%)	
Epithelial cell, hyperplasia	24 (48%)	25 (51%)	31 (66%)	22 (47%)
Thymocyte, atrophy	33 (66%)	33 (67%)	37 (79%)	34 (72%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Integumentary System				
Mammary gland	(43)	(47)	(48)	(47)
Galactocele	8 (19%)	9 (19%)	13 (27%)	8 (17%)
Hyperplasia		1 (2%)		
Inflammation, chronic			1 (2%)	
Mineralization, focal				1 (2%)
Pigmentation	1 (2%)	7 (15%)		5 (11%)
Epithelium, hyperplasia	1 (2%)		2 (4%)	2 (4%)
Skin	(49)	(50)	(50)	(50)
Fibrosis, focal	1 (2%)			
Hyperkeratosis				1 (2%)
Hyperplasia, basal cell				1 (2%)
Ulcer				1 (2%)
Artery, thrombosis				1 (2%)
Epidermis, hyperplasia			1 (2%)	
Lip, inflammation, chronic active		1 (2%)		
Lip, ulcer				1 (2%)
Subcutaneous tissue, inflammation, acute				1 (2%)
Subcutaneous tissue, inflammation, chronic active				1 (2%)
Vein, inflammation, acute				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Femur, fibrous osteodystrophy		17 (34%)	14 (28%)	12 (24%)
Femur, hyperostosis	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Joint, inflammation, chronic		1 (2%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hydrocephalus	1 (2%)	1 (2%)	1 (2%)	
Cerebellum, gliosis, focal				1 (2%)
Cerebellum, inflammation, acute, focal			1 (2%)	
Cerebrum, inflammation, acute			1 (2%)	
Cerebrum, necrosis			1 (2%)	
Choroid plexus, cerebrum, infiltration cellular, lymphocyte		1 (2%)		
Hippocampus, cerebrum, necrosis, focal	1 (2%)			
Meninges, hemorrhage			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	3 (6%)		1 (2%)	2 (4%)
Foreign body	1 (2%)			
Hemorrhage	1 (2%)	1 (2%)	3 (6%)	4 (8%)
Infiltration cellular, focal, histiocyte	2 (4%)			
Inflammation, chronic active	1 (2%)			
Alveolar epithelium, hyperplasia, focal	2 (4%)	1 (2%)	2 (4%)	
Artery, mineralization		1 (2%)	1 (2%)	

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Respiratory System (continued)				
Lung (continued)	(50)	(50)	(50)	(50)
Interstitium, inflammation, acute			1 (2%)	
Interstitium, inflammation, chronic	3 (6%)	8 (16%)	7 (14%)	6 (12%)
Interstitium, inflammation, chronic active		1 (2%)	1 (2%)	
Nose	(50)	(50)	(50)	(50)
Inflammation, acute	1 (2%)			1 (2%)
Inflammation, chronic	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic active	14 (28%)	12 (24%)	15 (30%)	16 (32%)
Metaplasia, focal, squamous			1 (2%)	2 (4%)
Metaplasia, squamous	1 (2%)	1 (2%)		
Nasolacrimal duct, inflammation, acute			1 (2%)	1 (2%)
Nasolacrimal duct, inflammation, chronic active	1 (2%)			1 (2%)
Trachea	(50)	(50)	(50)	(50)
Inflammation, chronic active		1 (2%)		
Glands, cyst, focal	1 (2%)			
Special Senses System				
Eye		(1)		(2)
Cornea, inflammation, chronic		1 (100%)		1 (50%)
Cornea, inflammation, chronic active				1 (50%)
Retina, atrophy		1 (100%)		1 (50%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	1 (2%)	3 (6%)	8 (16%)	10 (20%)
Hydronephrosis		1 (2%)		
Inflammation, acute			1 (2%)	
Mineralization, focal	1 (2%)			
Nephropathy, chronic	2 (4%)	1 (2%)	1 (2%)	
Bilateral, cyst		16 (32%)	13 (26%)	12 (24%)
Bilateral, inflammation, acute			1 (2%)	
Bilateral, mineralization, focal		2 (4%)	1 (2%)	1 (2%)
Bilateral, nephropathy, chronic	45 (90%)	48 (96%)	49 (98%)	50 (100%)
Bilateral, pelvis, transitional epithelium, hyperplasia	1 (2%)	21 (42%)	20 (40%)	17 (34%)
Bilateral, renal tubule, hyperplasia, focal		1 (2%)		
Bilateral, renal tubule, pigmentation	10 (20%)	5 (10%)	7 (14%)	6 (12%)
Pelvis, inflammation, acute				1 (2%)
Pelvis, transitional epithelium, hyperplasia	3 (6%)	10 (20%)	14 (28%)	12 (24%)
Renal tubule, hyperplasia, focal		5 (10%)	7 (14%)	2 (4%)
Renal tubule, hyperplasia, oncocytic				1 (2%)
Vein, inflammation, chronic			1 (2%)	
Vein, thrombosis			1 (2%)	
Urinary bladder	(49)	(50)	(50)	(50)
Hemorrhage	2 (4%)			
Inflammation, acute	1 (2%)	1 (2%)		
Inflammation, chronic active		1 (2%)	1 (2%)	
Transitional epithelium, hyperplasia			1 (2%)	

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

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

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	8	7	8
Natural deaths	11	4	11	4
Survivors				
Terminal sacrifice	30	38	32	38
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(48)	(49)	(49)	(49)
Intestine large, cecum	(45)	(49)	(47)	(48)
Intestine small, duodenum	(46)	(49)	(49)	(48)
Intestine small, jejunum	(44)	(49)	(47)	(48)
Intestine small, ileum	(47)	(49)	(45)	(49)
Liver	(50)	(50)	(50)	(49)
Hepatocellular carcinoma		1 (2%)		
Hepatocellular adenoma		1 (2%)	1 (2%)	1 (2%)
Hepatocellular adenoma, multiple		1 (2%)		
Mesentery	(12)	(7)	(6)	(7)
Carcinoma, metastatic, uterus	1 (8%)			
Oral mucosa		(3)		
Squamous cell papilloma		2 (67%)		
Pancreas	(49)	(49)	(49)	(46)
Carcinoma, metastatic, uterus	1 (2%)			
Acinus, adenoma				1 (2%)
Salivary glands	(50)	(50)	(50)	(49)
Stomach, forestomach	(49)	(50)	(50)	(49)
Stomach, glandular	(48)	(50)	(50)	(48)
Tongue	(2)	(3)		(2)
Squamous cell carcinoma	2 (100%)	1 (33%)		
Tooth	(1)		(2)	
Adamantinoma malignant	1 (100%)			
Cardiovascular System				
Heart	(50)	(50)	(50)	(49)
Schwannoma malignant			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(49)
Adenoma	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Adrenal medulla	(50)	(50)	(50)	(49)
Pheochromocytoma malignant		1 (2%)	1 (2%)	
Pheochromocytoma benign	2 (4%)	9 (18%)	9 (18%)	2 (4%)
Bilateral, pheochromocytoma benign	1 (2%)	2 (4%)		
Islets, pancreatic	(49)	(49)	(49)	(47)
Adenoma	2 (4%)		1 (2%)	1 (2%)
Carcinoma	3 (6%)			1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Endocrine System (continued)				
Pituitary gland	(50)	(49)	(50)	(47)
Pars distalis, adenoma	35 (70%)	32 (65%)	29 (58%)	25 (53%)
Pars distalis, carcinoma	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(48)
C-cell, adenoma	7 (14%)	5 (10%)	7 (14%)	7 (15%)
C-cell, carcinoma	1 (2%)	3 (6%)		2 (4%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(49)	(48)
Adenoma	9 (18%)	7 (14%)	3 (6%)	7 (15%)
Carcinoma			1 (2%)	1 (2%)
Bilateral, adenoma	1 (2%)		1 (2%)	
Ovary	(50)	(50)	(50)	(50)
Cystadenoma		1 (2%)		
Granulosa cell tumor malignant			1 (2%)	
Granulosa cell tumor benign		2 (4%)		
Granulosa-theca tumor benign		1 (2%)		
Bilateral, tubulostromal adenoma	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)			
Histiocytic sarcoma		1 (2%)		
Schwannoma malignant			1 (2%)	
Endometrium, polyp stromal	3 (6%)	7 (14%)	5 (10%)	4 (8%)
Endometrium, polyp stromal, multiple	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Endometrium, sarcoma stromal	1 (2%)			
Vagina		(1)		(1)
Schwannoma malignant				1 (100%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			1 (2%)
Lymph node	(7)	(11)	(9)	(9)
Mediastinal, carcinoma, metastatic, uterus	1 (14%)			
Lymph node, mandibular	(50)	(50)	(50)	(48)
Lymph node, mesenteric	(49)	(50)	(49)	(48)
Carcinoma, metastatic, uterus	1 (2%)			
Spleen	(50)	(50)	(50)	(49)
Fibrosarcoma				1 (2%)
Histiocytic sarcoma, metastatic, bone marrow	1 (2%)			
Thymus	(50)	(49)	(49)	(48)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Integumentary System				
Mammary gland	(50)	(50)	(48)	(49)
Adenoma	2 (4%)			
Carcinoma	3 (6%)			3 (6%)
Fibroadenoma	21 (42%)	25 (50%)	11 (23%)	19 (39%)
Fibroadenoma, multiple	9 (18%)		6 (13%)	5 (10%)
Skin	(50)	(50)	(49)	(50)
Lip, basal cell adenoma				1 (2%)
Pinna, squamous cell papilloma	1 (2%)			
Subcutaneous tissue, fibroma	1 (2%)	2 (4%)		1 (2%)
Musculoskeletal System				
Skeletal muscle		(3)	(3)	(2)
Sarcoma		1 (33%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Cerebellum, granular cell tumor malignant	1 (2%)			
Cerebrum, astrocytoma malignant				2 (4%)
Spinal cord	(1)	(2)	(2)	(2)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Alveolar/bronchiolar carcinoma		1 (2%)		
Carcinoma, metastatic, uterus	1 (2%)			
Histiocytic sarcoma, metastatic, bone marrow	1 (2%)			
Nose	(50)	(49)	(50)	(50)
Turbinate, adenoma	1 (2%)			
Special Senses System				
Zymbal's gland		(1)		(1)
Carcinoma		1 (100%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Pelvis, transitional epithelium, papilloma				1 (2%)
Renal tubule, adenoma				1 (2%)
Urinary bladder	(49)	(49)	(49)	(49)
Carcinoma, metastatic, uterus	1 (2%)			
Transitional epithelium, papilloma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)		1 (2%)
Leukemia mononuclear	21 (42%)	21 (42%)	17 (34%)	14 (28%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	50	50	49
Total primary neoplasms	138	133	98	108
Total animals with benign neoplasms	43	49	42	41
Total benign neoplasms	102	102	76	82
Total animals with malignant neoplasms	30	25	21	23
Total malignant neoplasms	36	31	22	26
Total animals with metastatic neoplasms	2			
Total metastatic neoplasms	8			

[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 Phenolphthalein: 0 ppm

Number of Days on Study	3 7 0	3 7 0	4 0 4	4 3 9	5 1 5	5 5 9	5 9 4	6 0 5	6 0 6	6 0 6	6 4 8	6 5 0	6 5 9	6 8 3	6 8 7	7 0 0	7 0 2	7 0 9	7 2 3	7 2 4	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	2 1 8	2 5 7	2 4 9	2 5 0	2 4 1	2 4 3	2 6 3	2 5 3	2 2 8	2 3 4	2 5 2	2 1 9	2 5 4	2 4 4	2 5 1	2 5 9	2 2 7	2 4 8	2 2 5	2 3 0	2 2 1	2 2 3	2 2 6	2 2 9	2 3 6
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	A	A	+	A	+	+	+	+	+	+	A	A	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	A	A	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	A	A	+	A	+	A	+	+	+	+	A	A	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery	+					+			+			+		+				+					+		
Carcinoma, metastatic, uterus																		X							
Pancreas	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																		X							
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue		+																	+						
Squamous cell carcinoma		X																	X						
Tooth			+																						
Adamantinoma malignant			X																						
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																						X			
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma														X											
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X					X	X						X	X	X	X			X	X	X	X	X	X	X
Pars distalis, carcinoma																		X							
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma													X			X					X			X	
C-cell, carcinoma																									
General Body System																									
None																									

+: 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 Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	2 3 8	2 3 9	2 4 0	2 4 5	2 4 6	2 5 5	2 5 8	2 6 0	2 6 2	2 1 6	2 1 7	2 2 0	2 2 2	2 2 4	2 3 1	2 3 2	2 3 3	2 3 5	2 3 7	2 4 2	2 4 7	2 5 6	2 6 1	2 6 4	2 6 5	Total Tissues/ Tumors
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery																					+	+	+	+	12
Carcinoma, metastatic, uterus																									1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, metastatic, uterus																									1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	48
Tongue																									2
Squamous cell carcinoma																									2
Tooth																									1
Adamantinoma malignant																									1
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																X							X		2
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																								X	2
Bilateral, pheochromocytoma benign																				X					1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																					X		X		2
Carcinoma			X		X																				3
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	M	+	M	+	M	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X	X	X	X	X	X	X	X	X	X		X	X	X		X		X	X	X	X		X	35
Pars distalis, carcinoma																									1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma	X			X						X															7
C-cell, carcinoma		X																							1
General Body System																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	3 7 0	3 7 0	4 0 4	4 3 9	5 1 5	5 5 9	5 9 4	6 0 5	6 0 6	6 0 6	6 4 8	6 5 0	6 5 9	6 8 3	6 8 7	7 0 0	7 0 2	7 0 9	7 2 3	7 2 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	2 1 8	2 5 7	2 4 9	2 5 0	2 4 1	2 4 3	2 6 3	2 5 3	2 2 8	2 3 4	2 5 2	2 1 9	2 5 4	2 4 4	2 5 1	2 5 9	2 7 7	2 4 8	2 3 5	2 3 0	2 2 1	2 2 3	2 2 6	2 3 9	2 3 6
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																			X						X
Bilateral, adenoma																					X				
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, tubulostromal adenoma	X																								
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																		X							
Endometrium, polyp stromal																X									
Endometrium, polyp stromal, multiple																							X		
Endometrium, sarcoma stromal									X																
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymph node					+			+					+	+			+	+							
Mediastinal, carcinoma, metastatic, uterus																		X							
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																		X							
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma, metastatic, bone marrow																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X															X			
Carcinoma							X																		
Fibroadenoma										X				X			X	X	X	X	X			X	
Fibroadenoma, multiple																									X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pinna, squamous cell papilloma																									
Subcutaneous tissue, fibroma											X														
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cerebellum, granular cell tumor malignant																								X	
Peripheral nerve						+																			
Spinal cord						+																			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	2 3 8	2 3 9	2 4 0	2 4 5	2 4 6	2 5 5	2 5 8	2 6 0	2 6 2	2 1 6	2 1 7	2 2 0	2 2 2	2 2 4	2 3 1	2 3 2	2 3 3	2 3 5	2 3 7	2 4 2	2 4 7	2 5 6	2 6 1	2 6 4	2 6 5	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X		X		X	X			X	X												X		9
Bilateral, adenoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, tubulostromal adenoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Endometrium, polyp stromal																		X					X			3
Endometrium, polyp stromal, multiple																										1
Endometrium, sarcoma stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma															X											1
Lymph node																	+									7
Mediastinal, carcinoma, metastatic, uterus																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, metastatic, uterus																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma, metastatic, bone marrow															X											1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										2
Carcinoma																				X					X	3
Fibroadenoma		X		X							X	X	X	X	X		X	X		X			X	X	X	21
Fibroadenoma, multiple	X				X	X		X		X									X		X	X				9
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pinna, squamous cell papilloma									X																	1
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cerebellum, granular cell tumor malignant																										1
Peripheral nerve																										1
Spinal cord																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	3 7 0	3 7 0	4 0 4	4 3 9	5 1 5	5 5 9	5 9 4	6 0 5	6 0 6	6 0 6	6 4 8	6 5 0	6 5 9	6 8 3	6 8 7	7 0 0	7 0 2	7 0 9	7 2 3	7 2 3	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	2 1 8	2 5 7	2 4 9	2 5 0	2 4 1	2 4 3	2 6 3	2 5 3	2 2 8	2 3 4	2 5 2	2 1 9	2 5 4	2 4 4	2 5 1	2 5 9	2 2 7	2 4 8	2 3 5	2 2 0	2 2 1	2 2 3	2 2 6	2 2 9	2 3 6
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																						X			
Carcinoma, metastatic, uterus																		X							
Histiocytic sarcoma, metastatic, bone marrow																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Turbinate, adenoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye							+															+			
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, uterus																		X							
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Leukemia mononuclear					X			X				X	X	X	X		X		X				X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	2 3 8	2 3 9	2 4 0	2 4 5	2 4 6	2 5 5	2 5 8	2 6 0	2 6 2	2 1 6	2 1 7	2 2 0	2 2 2	2 2 4	2 3 1	2 3 2	2 3 3	2 3 5	2 3 7	2 4 2	2 4 7	2 5 6	2 6 1	2 6 4	2 6 5	Total Tissues/ Tumors
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma										X							X								3
Carcinoma, metastatic, uterus																									1
Histiocytic sarcoma, metastatic, bone marrow															X										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Turbinate, adenoma													X												1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																									
Eye																									2
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, metastatic, uterus																									1
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma															X										1
Leukemia mononuclear					X	X		X			X		X				X	X	X	X			X	X	X	21

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm

Number of Days on Study	4 3 5	5 4 9	5 7 1	6 2 8	6 7 3	6 7 4	6 7 5	6 7 9	6 7 9	7 0 5	7 1 7	7 1 8	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	2 2 9 2	3 1 3	2 8 6	2 7 6	3 0 8	2 8 1	2 8 7	2 6 7	2 8 3	3 0 2	2 7 4	3 0 6	2 6 9	2 7 2	2 7 3	2 7 5	2 8 8	2 9 1	2 9 3	2 9 4	2 9 5	2 9 6	2 9 7	2 9 8	2 9 9
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma								X																
Hepatocellular adenoma																								
Hepatocellular adenoma, multiple										X														
Mesentery		+		+				+		+														
Oral mucosa											+													
Squamous cell papilloma											X													
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue														+										
Squamous cell carcinoma																								
Cardiovascular System																								
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																								
Pheochromocytoma benign		X					X					X			X									
Bilateral, pheochromocytoma benign										X									X					
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Pars distalis, adenoma			X	X			X		X				X	X	X		X	X		X		X	X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma								X																X
C-cell, carcinoma						X														X				
General Body System																								
Peritoneum																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm
(continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 0 0	3 0 1	3 0 3	3 0 4	3 0 5	3 0 7	3 0 9	3 1 0	3 1 1	3 1 2	3 1 4	3 1 5	3 2 6	3 2 6	3 2 8	3 2 0	3 2 1	3 2 7	3 2 8	3 2 9	3 2 0	3 2 2	3 2 4	3 2 5	3 2 9	3 2 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+		47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Hepatocellular carcinoma																										1
Hepatocellular adenoma										X																1
Hepatocellular adenoma, multiple																										1
Mesentery	+		+																			+				7
Oral mucosa										+									+							3
Squamous cell papilloma										X																2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Tongue																	+	+								3
Squamous cell carcinoma																		X								1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Adenoma			X																							1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Pheochromocytoma malignant																		X								1
Pheochromocytoma benign		X								X				X								X			X	9
Bilateral, pheochromocytoma benign																										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+		46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Pars distalis, adenoma	X	X			X	X	X	X	X		X	X	X	X	X	X			X	X	X	X			X	32
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
C-cell, adenoma																X	X						X			5
C-cell, carcinoma						X																				3
General Body System																										
Peritoneum																				+						1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm
(continued)

Number of Days on Study	4 3 5	5 4 9	5 7 1	6 2 8	6 7 3	6 7 4	6 7 5	6 7 9	6 7 9	7 0 5	7 1 7	7 1 8	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	2 9 2	3 1 3	2 8 6	2 7 6	3 0 8	2 8 1	2 8 7	2 6 7	2 8 3	3 0 2	2 7 4	3 0 6	2 6 9	2 7 2	2 7 3	2 7 5	2 8 8	2 9 1	2 9 3	2 9 4	2 9 5	2 9 6	2 9 7	2 9 8	2 9 9
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma										X				X	X	X				X					
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma									X																
Granulosa cell tumor benign																									
Granulosa-theca tumor benign																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Endometrium, polyp stromal	X											X			X						X				X
Endometrium, polyp stromal, multiple																									
Vagina	+																								
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+	+	+		+		+		+	+	+										+			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma	X				X				X				X	X						X		X		X	X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+						+															+	
Sarcoma																								X	
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve			+						+																
Spinal cord			+						+																
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																						X			
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Zymbal's gland																									
Carcinoma																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm
(continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 0 0	3 0 1	3 0 3	3 0 4	3 0 5	3 0 7	3 0 9	3 1 0	3 1 1	3 1 2	3 1 4	3 1 5	2 6 6	2 6 8	2 7 0	2 7 1	2 7 7	2 7 8	2 7 9	2 8 0	2 8 2	2 8 4	2 8 5	2 8 9	2 9 0	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X																			X			7
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma																										1
Granulosa cell tumor benign											X														X	2
Granulosa-theca tumor benign																			X							1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X																								1
Endometrium, polyp stromal													X								X					7
Endometrium, polyp stromal, multiple										X																1
Vagina																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node														+								+				11
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma	X	X		X	X			X	X		X	X	X	X			X	X		X	X	X		X		25
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma								X							X											2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										3
Sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										2
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma											X		X													3
Alveolar/bronchiolar carcinoma																		X								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																		+								1
Zymbal's gland																		+								1
Carcinoma																		X								1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm
(continued)

Number of Days on Study	4	5	5	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	3	4	7	2	7	7	7	7	7	0	1	1	3	3	3	3	3	3	3	3	3	3	3	3	3
	5	9	1	8	3	4	5	9	9	5	7	8	5	5	5	5	5	5	5	5	5	5	5	5	5
Carcass ID Number	2	3	2	2	3	2	2	2	2	3	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2
	9	1	8	7	0	8	8	6	8	0	7	0	6	7	7	7	8	9	9	9	9	9	9	9	9
	2	3	6	6	8	1	7	7	3	2	4	6	9	2	3	5	8	1	3	4	5	6	7	8	9
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Leukemia mononuclear		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 Phenolphthalein: 12,000 ppm
(continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 0 0	3 0 1	3 0 3	3 0 4	3 0 5	3 0 7	3 0 9	3 1 0	3 1 1	3 1 2	3 1 4	3 1 5	3 1 6	2 6 6	2 6 8	2 7 0	2 7 1	2 7 7	2 7 8	2 7 9	2 8 0	2 8 2	2 8 4	2 8 5	2 8 9	2 9 0	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X																								1
Leukemia mononuclear			X								X				X	X			X	X			X		X	21

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 25,000 ppm

Number of Days on Study	3 9 1	5 0 3	5 6 8	6 0 1	6 0 1 8	6 1 6	6 2 2	6 2 4	6 4 7	6 6 1	6 8 9	6 9 0	6 9 7	7 1 1	7 1 7	7 2 6	7 2 9	7 3 0	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 4 6	3 2	3 3 9	3 1 7	3 2 4	3 5 0	3 4 3	3 6 0	3 3 0	3 4 4	3 6 5	3 5 2	3 4 8	3 4 7	3 2 6	3 5 9	3 2 9	3 4 0	3 1 6	3 1 8	3 2 1	3 2 2	3 2 5	3 2 7	3 2 8
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	+	A	+	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery				+										+							+	+		+	
Pancreas	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth							+																		
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant			X																						
Pheochromocytoma benign					X		X				X							X							
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									X
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X	X				X			X			X			X				X		X	X	X	X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma															X			X	X						
General Body System																									
None																									
Genital System																									
Clitoral gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																						X			
Carcinoma																									
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant						X																			
Endometrium, polyp stromal													X							X					
Endometrium, polyp stromal, multiple																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 25,000 ppm
(continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 3 1	3 3 3	3 3 4	3 3 5	3 3 6	3 3 7	3 3 8	3 4 1	3 4 2	3 4 5	3 4 9	3 5 5	3 5 8	3 6 1	3 6 3	3 1 9	3 2 0	3 2 3	3 5 1	3 5 3	3 5 4	3 5 6	3 5 7	3 6 2	3 6 4	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma												X														1
Mesentery																				+						6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth															+											2
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																								X		1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma										X																1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign						X						X				X					X		X			9
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma				X		X	X			X	X	X	X	X	X		X	X	X	X	X	X	X		X	29
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma					X														X	X		X				7
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma	X																			X						3
Carcinoma														X												1
Bilateral, adenoma																		X								1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor malignant																								X		1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																										1
Endometrium, polyp stromal									X				X								X					5
Endometrium, polyp stromal, multiple																							X			1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 25,000 ppm
(continued)

Number of Days on Study	3 9 1	5 0 3	5 6 8	6 0 1	6 0 8	6 1 6	6 2 2	6 2 4	6 4 7	6 6 1	6 8 9	6 9 0	6 9 7	7 1 1	7 1 7	7 2 6	7 2 9	7 3 0	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 4 6	3 2	3 9	3 1 7	3 2 4	3 5 0	3 4 3	3 6 0	3 3 0	3 4 4	3 6 5	3 5 2	3 4 8	3 4 7	3 2 6	3 5 9	3 2 9	3 4 0	3 1 6	3 1 8	3 2 1	3 2 2	3 2 5	3 2 7	3 2 8
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+		+				+		+	+					+	+	+							
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Integumentary System																									
Mammary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																			X	X	X				
Fibroadenoma, multiple																	X	X							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle		+										+			+										
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve												+			+										
Spinal cord												+			+										
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																						X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																							+	+	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				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 Phenolphthalein: 25,000 ppm
(continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 3 1	3 3 3	3 3 4	3 3 5	3 3 6	3 3 7	3 3 8	3 4 1	4 4 2	4 4 5	4 4 9	5 5 5	5 5 8	6 6 1	6 6 3	1 1 9	2 2 0	2 2 3	5 5 1	5 5 3	5 5 4	5 5 6	5 5 7	6 6 2	6 6 4	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node																+										9
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibroadenoma		X			X						X					X	X	X					X		X	11
Fibroadenoma, multiple			X							X											X	X				6
Skin	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										3
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										2
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X			X	X		X		X				X					X					17

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 50,000 ppm

Number of Days on Study	2 7 1	3 3 0	4 3 4	4 8 4	4 9 1	5 0 6	5 1 2	5 8 1	6 2 2	6 9 7	7 1 2	7 2 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 6 7	3 9 3	3 8 7	4 1 0	4 0 8	3 7 8	3 7 6	4 1 4	3 9 0	3 9 7	3 7 4	3 9 8	3 6 6	3 6 8	3 6 9	3 7 0	3 7 3	3 7 5	3 7 7	3 7 9	3 8 2	3 8 9	3 9 1	3 9 2	3 9 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																								X	
Mesentery	+				+				+											+	+				+
Pancreas	+	+	M	+	+	+	+	+	M	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																						X			
Salivary glands	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	A	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																				X	X				
Islets, pancreatic	+	+	+	+	+	+	+	+	M	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	M
Pituitary gland	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Pars distalis, adenoma						X		X						X	X	X		X				X	X	X	
Thyroid gland	+	+	A	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																		X			X		X		
C-cell, carcinoma																			X	X					
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X							X			X
Carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oviduct																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal										X															
Endometrium, polyp stromal, multiple																									
Vagina					+																				
Schwannoma malignant					X																				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 50,000 ppm
(continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 9 5	3 9 6	3 9 9	4 0 0	4 0 1	4 0 2	4 0 3	4 0 4	4 0 5	4 0 6	4 0 7	4 0 9	4 1 1	4 1 2	4 1 3	4 1 5	3 7 1	3 7 2	3 8 0	3 8 1	3 8 3	3 8 4	3 8 5	3 8 6	3 8 8	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma																										1
Mesentery																		+								7
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Acinus, adenoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue												+									+					2
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma					X																X					2
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma						X																				1
Carcinoma						X																				1
Parathyroid gland	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pars distalis, adenoma	X	X	X				X	X	X	X		X			X	X	X	X		X	X	X		X		25
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma		X	X															X						X		7
C-cell, carcinoma																										2
General Body System																										
None																										
Genital System																										
Clitoral gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma			X						X		X													X		7
Carcinoma									X																	1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oviduct																		+								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal	X												X			X										4
Endometrium, polyp stromal, multiple																	X									1
Vagina																										1
Schwannoma malignant																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 50,000 ppm
(continued)

Number of Days on Study	2 7 1	3 3 0	4 3 4	4 8 4	4 9 1	5 0 6	5 1 2	5 8 1	6 2 2	6 9 7	7 1 2	7 2 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5
Carcass ID Number	3 6 7	3 9 3	3 8 7	4 1 0	4 0 8	3 7 8	3 7 6	4 1 4	3 9 0	3 9 7	3 7 4	3 9 8	3 6 6	3 6 8	3 6 9	3 7 0	3 7 3	3 7 5	3 7 7	3 7 9	3 8 2	3 8 9	3 9 1	3 9 2	3 9 4
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymph node				+	+		+		+	+		+					+								
Lymph node, mandibular	+	+	A	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Carcinoma		X				X								X											
Fibroadenoma						X			X		X		X			X	X		X		X		X		
Fibroadenoma, multiple														X								X			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lip, basal cell adenoma																									
Subcutaneous tissue, fibroma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+			+																					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cerebrum, astrocytoma malignant	X																								
Peripheral nerve	+			+																					
Spinal cord	+			+																					
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Zymbal's gland												+													
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pelvis, transitional epithelium, papilloma																									
Renal tubule, adenoma																									
Ureter																									
Urinary bladder	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma						X																			
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Leukemia mononuclear			X	X			X		X	X		X				X			X		X				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Phenolphthalein: 50,000 ppm
(continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	3 9 5	3 9 6	3 9 9	4 0 0	4 0 1	4 0 2	4 0 3	4 0 4	4 0 5	4 0 6	4 0 7	4 0 9	4 1 1	4 1 2	4 1 3	4 1 5	3 7 1	3 7 2	3 8 0	3 8 1	3 8 3	3 8 4	3 8 5	3 8 6	3 8 8	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																							X			1
Lymph node					+	+																				9
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrosarcoma																X										1
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										3
Fibroadenoma		X						X	X		X				X		X			X		X	X		X	19
Fibroadenoma, multiple							X			X				X												5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lip, basal cell adenoma												X														1
Subcutaneous tissue, fibroma																		X								1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cerebrum, astrocytoma malignant																								X		2
Peripheral nerve																										2
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																						X		X		2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Zymbal's gland																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pelvis, transitional epithelium, papilloma		X																								1
Renal tubule, adenoma																									X	1
Ureter																								+		1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Transitional epithelium, papilloma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																							X			1
Leukemia mononuclear		X				X												X	X		X		X			14

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

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	3/50 (6%)	11/50 (22%)	9/50 (18%)	2/49 (4%)
Adjusted rate[b]	10.0%	25.8%	23.7%	5.3%
Terminal rate[c]	3/30 (10%)	7/38 (18%)	5/32 (16%)	2/38 (5%)
First incidence (days)	735 (T)	549	608	735 (T)
Life table test[d]	P=0.138N	P=0.060	P=0.088	P=0.392N
Logistic regression test[d]	P=0.197N	P=0.031	P=0.074	P=0.392N
Cochran-Armitage test[d]	P=0.207N			
Fisher exact test[d]		P=0.020	P=0.061	P=0.510N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	3/50 (6%)	12/50 (24%)	10/50 (20%)	2/49 (4%)
Adjusted rate	10.0%	28.2%	25.3%	5.3%
Terminal rate	3/30 (10%)	8/38 (21%)	5/32 (16%)	2/38 (5%)
First incidence (days)	735 (T)	549	568	735 (T)
Life table test	P=0.130N	P=0.040	P=0.058	P=0.392N
Logistic regression test	P=0.189N	P=0.019	P=0.041	P=0.392N
Cochran-Armitage test	P=0.195N			
Fisher exact test		P=0.011	P=0.036	P=0.510N
Clitoral Gland: Adenoma				
Overall rate	10/50 (20%)	7/50 (14%)	4/49 (8%)	7/48 (15%)
Adjusted rate	32.2%	17.8%	12.5%	18.9%
Terminal rate	9/30 (30%)	6/38 (16%)	4/32 (13%)	7/37 (19%)
First incidence (days)	723	705	735 (T)	735 (T)
Life table test	P=0.162N	P=0.142N	P=0.053N	P=0.150N
Logistic regression test	P=0.176N	P=0.157N	P=0.044N	P=0.162N
Cochran-Armitage test	P=0.275N			
Fisher exact test		P=0.298N	P=0.080N	P=0.330N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	10/50 (20%)	7/50 (14%)	5/49 (10%)	7/48 (15%)
Adjusted rate	32.2%	17.8%	15.6%	18.9%
Terminal rate	9/30 (30%)	6/38 (16%)	5/32 (16%)	7/37 (19%)
First incidence (days)	723	705	735 (T)	735 (T)
Life table test	P=0.172N	P=0.142N	P=0.097N	P=0.150N
Logistic regression test	P=0.186N	P=0.157N	P=0.083N	P=0.162N
Cochran-Armitage test	P=0.291N			
Fisher exact test		P=0.298N	P=0.140N	P=0.330N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	1/49 (2%)
Adjusted rate	0.0%	7.2%	3.1%	2.6%
Terminal rate	0/30 (0%)	1/38 (3%)	1/32 (3%)	1/38 (3%)
First incidence (days)	—[e]	679	735 (T)	735 (T)
Life table test	P=0.596N	P=0.162	P=0.513	P=0.547
Logistic regression test	P=0.602	P=0.126	P=0.513	P=0.547
Cochran-Armitage test	P=0.592			
Fisher exact test		P=0.121	P=0.500	P=0.495

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/50 (6%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	10.0%	7.9%	3.1%	5.3%
Terminal rate	3/30 (10%)	3/38 (8%)	1/32 (3%)	2/38 (5%)
First incidence (days)	735 (T)	735 (T)	735 (T)	735 (T)
Life table test	P=0.276N	P=0.550N	P=0.281N	P=0.392N
Logistic regression test	P=0.276N	P=0.550N	P=0.281N	P=0.392N
Cochran-Armitage test	P=0.341N			
Fisher exact test		P=0.661N	P=0.309N	P=0.500N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	3/50 (6%)	4/50 (8%)	1/50 (2%)	2/50 (4%)
Adjusted rate	10.0%	10.5%	3.1%	5.3%
Terminal rate	3/30 (10%)	4/38 (11%)	1/32 (3%)	2/38 (5%)
First incidence (days)	735 (T)	735 (T)	735 (T)	735 (T)
Life table test	P=0.225N	P=0.629	P=0.281N	P=0.392N
Logistic regression test	P=0.225N	P=0.630	P=0.281N	P=0.392N
Cochran-Armitage test	P=0.288N			
Fisher exact test		P=0.500	P=0.309N	P=0.500N
Mammary Gland: Fibroadenoma				
Overall rate	30/50 (60%)	25/50 (50%)	17/50 (34%)	24/50 (48%)
Adjusted rate	83.2%	60.6%	50.0%	58.4%
Terminal rate	24/30 (80%)	22/38 (58%)	15/32 (47%)	21/38 (55%)
First incidence (days)	606	435	729	506
Life table test	P=0.026N	P=0.019N	P=0.002N	P=0.014N
Logistic regression test	P=0.060N	P=0.050N	P<0.001N	P=0.075N
Cochran-Armitage test	P=0.125N			
Fisher exact test		P=0.211N	P=0.008N	P=0.158N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	32/50 (64%)	25/50 (50%)	17/50 (34%)	24/50 (48%)
Adjusted rate	86.3%	60.6%	50.0%	58.4%
Terminal rate	25/30 (83%)	22/38 (58%)	15/32 (47%)	21/38 (55%)
First incidence (days)	594	435	729	506
Life table test	P=0.011N	P=0.007N	P<0.001N	P=0.005N
Logistic regression test	P=0.047N	P=0.021N	P<0.001N	P=0.033N
Cochran-Armitage test	P=0.070N			
Fisher exact test		P=0.113N	P=0.002N	P=0.079N
Mammary Gland: Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	8.8%	0.0%	0.0%	6.7%
Terminal rate	2/30 (7%)	0/38 (0%)	0/32 (0%)	1/38 (3%)
First incidence (days)	594	—	—	330
Life table test	P=0.461	P=0.093N	P=0.112N	P=0.612N
Logistic regression test	P=0.492	P=0.122N	P=0.120N	P=0.662N
Cochran-Armitage test	P=0.438			
Fisher exact test		P=0.121N	P=0.121N	P=0.661N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Mammary Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	12.0%	0.0%	0.0%	6.7%
Terminal rate	3/30 (10%)	0/38 (0%)	0/32 (0%)	1/38 (3%)
First incidence (days)	594	—	—	330
Life table test	P=0.554N	P=0.043N	P=0.056N	P=0.435N
Logistic regression test	P=0.542N	P=0.058N	P=0.059N	P=0.500N
Cochran-Armitage test	P=0.584N			
Fisher exact test		P=0.059N	P=0.059N	P=0.500N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	32/50 (64%)	25/50 (50%)	17/50 (34%)	25/50 (50%)
Adjusted rate	86.3%	60.6%	50.0%	59.2%
Terminal rate	25/30 (83%)	22/38 (58%)	15/32 (47%)	21/38 (55%)
First incidence (days)	594	435	729	330
Life table test	P=0.021N	P=0.007N	P<0.001N	P=0.009N
Logistic regression test	P=0.043N	P=0.021N	P<0.001N	P=0.073N
Cochran-Armitage test	P=0.103N			
Fisher exact test		P=0.113N	P=0.002N	P=0.113N
Oral Cavity (Oral Mucosa or Tongue): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	5.1%	7.6%	0.0%	0.0%
Terminal rate	0/30 (0%)	2/38 (5%)	0/32 (0%)	0/38 (0%)
First incidence (days)	370	717	—	—
Life table test	P=0.061N	P=0.577	P=0.230N	P=0.222N
Logistic regression test	P=0.070N	P=0.408	P=0.343N	P=0.216N
Cochran-Armitage test	P=0.071N			
Fisher exact test		P=0.500	P=0.247N	P=0.247N
Pancreatic Islets: Carcinoma				
Overall rate	3/49 (6%)	0/49 (0%)	0/49 (0%)	1/47 (2%)
Adjusted rate	9.2%	0.0%	0.0%	2.6%
Terminal rate	2/30 (7%)	0/38 (0%)	0/32 (0%)	1/38 (3%)
First incidence (days)	683	—	—	735 (T)
Life table test	P=0.222N	P=0.091N	P=0.111N	P=0.238N
Logistic regression test	P=0.254N	P=0.101N	P=0.110N	P=0.297N
Cochran-Armitage test	P=0.261N			
Fisher exact test		P=0.121N	P=0.121N	P=0.324N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	5/49 (10%)	0/49 (0%)	1/49 (2%)	1/47 (2%)
Adjusted rate	15.7%	0.0%	3.1%	2.6%
Terminal rate	4/30 (13%)	0/38 (0%)	1/32 (3%)	1/38 (3%)
First incidence (days)	683	—	735 (T)	735 (T)
Life table test	P=0.069N	P=0.019N	P=0.089N	P=0.063N
Logistic regression test	P=0.084N	P=0.022N	P=0.086N	P=0.091N
Cochran-Armitage test	P=0.095N			
Fisher exact test		P=0.028N	P=0.102N	P=0.112N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	35/50 (70%)	32/49 (65%)	29/50 (58%)	25/47 (53%)
Adjusted rate	89.5%	77.7%	75.6%	63.9%
Terminal rate	26/30 (87%)	28/37 (76%)	23/32 (72%)	23/37 (62%)
First incidence (days)	370	571	391	506
Life table test	P=0.002N	P=0.040N	P=0.078N	P=0.002N
Logistic regression test	P=0.032N	P=0.154N	P=0.090N	P=0.034N
Cochran-Armitage test	P=0.044N			
Fisher exact test		P=0.388N	P=0.149N	P=0.067N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	36/50 (72%)	32/49 (65%)	29/50 (58%)	25/47 (53%)
Adjusted rate	89.8%	77.7%	75.6%	63.9%
Terminal rate	26/30 (87%)	28/37 (76%)	23/32 (72%)	23/37 (62%)
First incidence (days)	370	571	391	506
Life table test	P=0.001N	P=0.027N	P=0.055N	P<0.001N
Logistic regression test	P=0.021N	P=0.102N	P=0.057N	P=0.020N
Cochran-Armitage test	P=0.030N			
Fisher exact test		P=0.308N	P=0.104N	P=0.044N
Thyroid Gland (C-cell): Adenoma				
Overall rate	7/50 (14%)	5/50 (10%)	7/50 (14%)	7/48 (15%)
Adjusted rate	21.2%	12.6%	20.5%	18.4%
Terminal rate	5/30 (17%)	4/38 (11%)	5/32 (16%)	7/38 (18%)
First incidence (days)	659	679	717	735 (T)
Life table test	P=0.524N	P=0.241N	P=0.557N	P=0.440N
Logistic regression test	P=0.493	P=0.293N	P=0.549N	P=0.561N
Cochran-Armitage test	P=0.435			
Fisher exact test		P=0.380N	P=0.613N	P=0.581
Thyroid Gland (C-cell): Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	2/48 (4%)
Adjusted rate	3.3%	7.4%	0.0%	5.3%
Terminal rate	1/30 (3%)	2/38 (5%)	0/32 (0%)	2/38 (5%)
First incidence (days)	735 (T)	674	—	735 (T)
Life table test	P=0.606N	P=0.390	P=0.487N	P=0.583
Logistic regression test	P=0.568	P=0.346	P=0.487N	P=0.583
Cochran-Armitage test	P=0.547			
Fisher exact test		P=0.309	P=0.500N	P=0.485
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	8/50 (16%)	8/50 (16%)	7/50 (14%)	9/48 (19%)
Adjusted rate	24.3%	19.6%	20.5%	23.7%
Terminal rate	6/30 (20%)	6/38 (16%)	5/32 (16%)	9/38 (24%)
First incidence (days)	659	674	717	735 (T)
Life table test	P=0.520N	P=0.419N	P=0.442N	P=0.514N
Logistic regression test	P=0.471	P=0.503N	P=0.429N	P=0.574
Cochran-Armitage test	P=0.410			
Fisher exact test		P=0.607N	P=0.500N	P=0.463

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Uterus: Stromal Polyp				
Overall rate	4/50 (8%)	8/50 (16%)	6/50 (12%)	5/50 (10%)
Adjusted rate	12.6%	19.6%	17.8%	12.7%
Terminal rate	3/30 (10%)	6/38 (16%)	5/32 (16%)	4/38 (11%)
First incidence (days)	700	435	697	697
Life table test	P=0.431N	P=0.299	P=0.412	P=0.632
Logistic regression test	P=0.533N	P=0.197	P=0.424	P=0.558
Cochran-Armitage test	P=0.539N			
Fisher exact test		P=0.178	P=0.370	P=0.500
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	5/50 (10%)	8/50 (16%)	6/50 (12%)	5/50 (10%)
Adjusted rate	14.7%	19.6%	17.8%	12.7%
Terminal rate	3/30 (10%)	6/38 (16%)	5/32 (16%)	4/38 (11%)
First incidence (days)	606	435	697	697
Life table test	P=0.338N	P=0.422	P=0.547	P=0.504N
Logistic regression test	P=0.434N	P=0.277	P=0.547	P=0.606N
Cochran-Armitage test	P=0.437N			
Fisher exact test		P=0.277	P=0.500	P=0.630N
All Organs: Mononuclear Cell Leukemia				
Overall rate	21/50 (42%)	21/50 (42%)	17/50 (34%)	14/50 (28%)
Adjusted rate	54.3%	44.4%	39.8%	31.4%
Terminal rate	13/30 (43%)	12/38 (32%)	7/32 (22%)	8/38 (21%)
First incidence (days)	515	549	601	434
Life table test	P=0.040N	P=0.285N	P=0.212N	P=0.046N
Logistic regression test	P=0.107N	P=0.571N	P=0.224N	P=0.101N
Cochran-Armitage test	P=0.058N			
Fisher exact test		P=0.580N	P=0.268N	P=0.104N
All Organs: Benign Neoplasms				
Overall rate	43/50 (86%)	49/50 (98%)	42/50 (84%)	41/50 (82%)
Adjusted rate	100.0%	100.0%	95.4%	95.3%
Terminal rate	30/30 (100%)	38/38 (100%)	30/32 (94%)	36/38 (95%)
First incidence (days)	370	435	391	506
Life table test	P=0.014N	P=0.289N	P=0.281N	P=0.016N
Logistic regression test	P=0.126N	P=0.147	P=0.301N	P=0.335N
Cochran-Armitage test	P=0.111N			
Fisher exact test		P=0.030	P=0.500N	P=0.393N
All Organs: Malignant Neoplasms				
Overall rate	30/50 (60%)	25/50 (50%)	21/50 (42%)	23/50 (46%)
Adjusted rate	69.0%	52.9%	47.0%	47.5%
Terminal rate	17/30 (57%)	16/38 (42%)	9/32 (28%)	13/38 (34%)
First incidence (days)	370	549	568	271
Life table test	P=0.071N	P=0.066N	P=0.058N	P=0.055N
Logistic regression test	P=0.096N	P=0.239N	P=0.061N	P=0.127N
Cochran-Armitage test	P=0.103N			
Fisher exact test		P=0.211N	P=0.055N	P=0.115N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	50/50 (100%)	50/50 (100%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%	98.0%
Terminal rate	30/30 (100%)	38/38 (100%)	32/32 (100%)	37/38 (97%)
First incidence (days)	370	435	391	271
Life table test	P=0.131N	P=0.073N	P=0.401N	P=0.086N
Logistic regression test	P=0.627N	P=0.834	P=0.716	P=0.758
Cochran-Armitage test	P=0.591N			
Fisher exact test		P=0.500	P=0.500	P=0.753N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, liver, lung, pancreatic islets, pituitary gland, thyroid gland, and uterus; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, 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 Adrenal Medulla Pheochromocytoma in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Benign	Malignant	Benign or Malignant
Historical Incidence at EG&G Mason Research Institute/TSI Mason Laboratories, Inc.			
1-Amino-2,4-dibromoanthraquinone	2/47	0/47	2/47
Acetaminophen	3/39	0/39	3/39
HC Yellow 4	1/49	1/49	2/49
Methylphenidate Hydrochloride	3/50	0/50	3/50
Pentaerythritol Tetranitrate	2/49	0/49	2/49
Quercetin	3/49	1/49	3/49
Turmeric Oleoresin	3/50	0/50	3/50
Overall Historical Incidence			
Total	50/1,274 (3.9%)	6/1,274 (0.5%)	64/1,274 (5.0%)
Standard deviation	2.4%	0.9%	2.7%
Range	0%-8%	0%-2%	2%-12%

[a] Data as of 12 May 1995; includes data for complex and unspecified pheochromocytoma

TABLE B4b
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 EG&G Mason Research Institute/TSI Mason Laboratories, Inc.			
1-Amino-2,4-dibromoanthraquinone	0/50	0/50	0/50
Acetaminophen	0/50	0/50	0/50
HC Yellow 4	0/49	0/49	0/49
Methylphenidate Hydrochloride	0/50	0/50	0/50
Pentaerythritol Tetranitrate	0/50	0/50	0/50
Quercetin	0/49	0/49	0/49
Turmeric Oleoresin	0/50	0/50	0/50
Overall Historical Incidence			
Total	0/1,298 (0.0%)	1/1,298 (0.1%)	1/1,298 (0.1%)
Standard deviation		0.4%	0.4%
Range		0%-2%	0%-2%

[a] Data as of 12 May 1995

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

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	8	7	8
Natural deaths	11	4	11	4
Survivors				
Terminal sacrifice	30	38	32	38
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(48)	(49)	(49)	(49)
Parasite metazoan	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Intestine large, rectum	(49)	(47)	(48)	(49)
Edema	1 (2%)			
Inflammation, acute	1 (2%)			
Parasite metazoan	4 (8%)			
Intestine large, cecum	(45)	(49)	(47)	(48)
Edema	1 (2%)	3 (6%)		
Necrosis	1 (2%)			
Intestine small, duodenum	(46)	(49)	(49)	(48)
Inflammation, chronic		1 (2%)		
Intestine small, jejunum	(44)	(49)	(47)	(48)
Ulcer, focal				1 (2%)
Intestine small, ileum	(47)	(49)	(45)	(49)
Necrosis			1 (2%)	
Ulcer, focal				1 (2%)
Liver	(50)	(50)	(50)	(49)
Angiectasis, focal	1 (2%)	4 (8%)	4 (8%)	
Basophilic focus	32 (64%)	34 (68%)	34 (68%)	34 (69%)
Clear cell focus	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Cytoplasmic alteration, focal		1 (2%)		
Eosinophilic focus	12 (24%)	12 (24%)	12 (24%)	4 (8%)
Fibrosis, focal	1 (2%)			
Hematopoietic cell proliferation	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Hepatodiaphragmatic nodule	3 (6%)	7 (14%)	6 (12%)	4 (8%)
Hyperplasia, focal				1 (2%)
Infiltration cellular, focal, lymphocyte		1 (2%)		
Inflammation, chronic	7 (14%)	3 (6%)	4 (8%)	4 (8%)
Mixed cell focus	25 (50%)	23 (46%)	31 (62%)	33 (67%)
Necrosis	2 (4%)		1 (2%)	
Bile duct, hyperplasia, focal	1 (2%)			
Centrilobular, necrosis	1 (2%)			
Hepatocyte, vacuolization cytoplasmic	3 (6%)	1 (2%)	3 (6%)	6 (12%)
Kupffer cell, hyperplasia, diffuse	1 (2%)			
Mesentery	(12)	(7)	(6)	(7)
Accessory spleen	1 (8%)			1 (14%)
Fat, necrosis	10 (83%)	7 (100%)	6 (100%)	5 (71%)
Oral mucosa		(3)		
Ulcer		1 (33%)		

[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 Phenolphthalein
(continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Alimentary System (continued)				
Pancreas	(49)	(49)	(49)	(46)
Acinus, atrophy, diffuse	4 (8%)	1 (2%)	1 (2%)	2 (4%)
Acinus, atrophy, focal	17 (35%)	28 (57%)	30 (61%)	22 (48%)
Acinus, basophilic focus		1 (2%)		
Acinus, hyperplasia, focal			2 (4%)	1 (2%)
Salivary glands	(50)	(50)	(50)	(49)
Atrophy, diffuse		1 (2%)		
Infiltration cellular, focal, lymphocyte		1 (2%)		
Parotid gland, atrophy, diffuse	2 (4%)			
Parotid gland, atrophy, focal		1 (2%)	2 (4%)	1 (2%)
Submandibular gland, atrophy, diffuse	1 (2%)			
Stomach, forestomach	(49)	(50)	(50)	(49)
Inflammation, chronic	1 (2%)	2 (4%)	1 (2%)	
Ulcer, focal			3 (6%)	1 (2%)
Epithelium, hyperplasia	4 (8%)	3 (6%)	4 (8%)	2 (4%)
Stomach, glandular	(48)	(50)	(50)	(48)
Erosion, focal	6 (13%)	2 (4%)	1 (2%)	2 (4%)
Inflammation, chronic			1 (2%)	
Glands, cyst, focal	8 (17%)	9 (18%)	21 (42%)	4 (8%)
Serosa, inflammation, acute			1 (2%)	
Tongue	(2)	(3)		(2)
Capillary, erosion, focal		1 (33%)		1 (50%)
Tooth	(1)		(2)	
Developmental malformation			1 (50%)	
Gingiva, inflammation, chronic			1 (50%)	
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, media, mineralization	1 (2%)			
Heart	(50)	(50)	(50)	(49)
Cardiomyopathy	36 (72%)	32 (64%)	34 (68%)	27 (55%)
Artery, inflammation, chronic	1 (2%)			
Atrium, thrombosis	2 (4%)			
Myocardium, inflammation, acute, focal			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(49)
Hematopoietic cell proliferation				1 (2%)
Hemorrhage	1 (2%)			
Hyperplasia, focal	2 (4%)	5 (10%)	5 (10%)	6 (12%)
Hypertrophy, focal	2 (4%)	1 (2%)	3 (6%)	3 (6%)
Inflammation, acute, focal				1 (2%)
Pigmentation	1 (2%)			
Vacuolization cytoplasmic, focal	6 (12%)	17 (34%)	18 (37%)	10 (20%)
Bilateral, hematopoietic cell proliferation		1 (2%)		
Bilateral, hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Bilateral, necrosis	2 (4%)	2 (4%)	1 (2%)	
Bilateral, vacuolization cytoplasmic, focal			2 (4%)	1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Phenolphthalein
(continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Endocrine System (continued)				
Adrenal medulla	(50)	(50)	(50)	(49)
Hyperplasia, focal	9 (18%)	14 (28%)	15 (30%)	11 (22%)
Infiltration cellular, lymphocyte		1 (2%)		
Bilateral, hyperplasia, focal	1 (2%)	4 (8%)		
Islets, pancreatic	(49)	(49)	(49)	(47)
Metaplasia, focal	1 (2%)	1 (2%)		
Parathyroid gland	(46)	(46)	(47)	(44)
Hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	
Pituitary gland	(50)	(49)	(50)	(47)
Pars distalis, angiectasis, focal	1 (2%)	1 (2%)	1 (2%)	5 (11%)
Pars distalis, cyst	14 (28%)	17 (35%)	16 (32%)	11 (23%)
Pars distalis, hyperplasia, focal	5 (10%)	2 (4%)	2 (4%)	4 (9%)
Pars distalis, pigmentation	1 (2%)		1 (2%)	
Rathke's cleft, cyst		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(48)
Ultimobranchial cyst		1 (2%)		2 (4%)
C-cell, hyperplasia, diffuse	1 (2%)			
C-cell, hyperplasia, focal	20 (40%)	18 (36%)	17 (34%)	9 (19%)
Follicle, cyst	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Follicle, hyperplasia, focal		1 (2%)		
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(49)	(48)
Cyst		1 (2%)	1 (2%)	
Hyperplasia, focal	1 (2%)	4 (8%)	4 (8%)	3 (6%)
Inflammation, chronic	4 (8%)	4 (8%)	8 (16%)	7 (15%)
Inflammation, chronic active	3 (6%)	3 (6%)	1 (2%)	5 (10%)
Bilateral, atrophy	1 (2%)	2 (4%)		
Bilateral, hyperplasia, focal			1 (2%)	
Duct, hyperplasia, focal	1 (2%)			
Duct, inflammation, acute		1 (2%)		
Ovary	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Bilateral, atrophy				1 (2%)
Bilateral, cyst				1 (2%)
Bilateral, pigmentation		1 (2%)		
Bursa, cyst	1 (2%)	4 (8%)	4 (8%)	6 (12%)
Follicle, cyst		2 (4%)	2 (4%)	4 (8%)
Periovarian tissue, cyst		1 (2%)		
Oviduct				(1)
Cyst				1 (100%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Phenolphthalein
(continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Genital System (continued)				
Uterus	(50)	(50)	(50)	(50)
Hydrometra	4 (8%)	4 (8%)	3 (6%)	6 (12%)
Inflammation, chronic active		1 (2%)		
Necrosis		1 (2%)		
Thrombosis				1 (2%)
Endometrium, atrophy	1 (2%)			
Endometrium, cyst		1 (2%)	1 (2%)	4 (8%)
Endometrium, inflammation, acute	2 (4%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)	3 (6%)	5 (10%)
Hyperplasia	6 (12%)	9 (18%)	4 (8%)	6 (12%)
Myelofibrosis	5 (10%)	1 (2%)	2 (4%)	2 (4%)
Lymph node	(7)	(11)	(9)	(9)
Iliac, hemorrhage			1 (11%)	
Iliac, hyperplasia, lymphoid			1 (11%)	
Mediastinal, hemorrhage	1 (14%)			1 (11%)
Mediastinal, pigmentation				1 (11%)
Renal, infiltration cellular, histiocyte	1 (14%)			
Lymph node, mandibular	(50)	(50)	(50)	(48)
Degeneration, cystic	6 (12%)	11 (22%)	2 (4%)	8 (17%)
Hemorrhage	2 (4%)			1 (2%)
Hyperplasia, lymphoid	14 (28%)	4 (8%)	6 (12%)	5 (10%)
Infiltration cellular, histiocyte	4 (8%)	3 (6%)	1 (2%)	3 (6%)
Necrosis	1 (2%)			
Pigmentation	1 (2%)	1 (2%)		1 (2%)
Lymph node, mesenteric	(49)	(50)	(49)	(48)
Degeneration, cystic	2 (4%)	1 (2%)	4 (8%)	1 (2%)
Ectasia	1 (2%)			
Hemorrhage	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Hyperplasia, lymphoid	2 (4%)			1 (2%)
Infiltration cellular, histiocyte	30 (61%)	33 (66%)	31 (63%)	26 (54%)
Pigmentation		1 (2%)		
Spleen	(50)	(50)	(50)	(49)
Congestion				1 (2%)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation	9 (18%)	7 (14%)	6 (12%)	6 (12%)
Pigmentation	8 (16%)	7 (14%)	10 (20%)	6 (12%)
Lymphoid follicle, atrophy	3 (6%)			
Thymus	(50)	(49)	(49)	(48)
Cyst		1 (2%)	1 (2%)	
Hemorrhage	1 (2%)		1 (2%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)			
Epithelial cell, hyperplasia	20 (40%)	34 (69%)	31 (63%)	23 (48%)
Thymocyte, atrophy	21 (42%)	19 (39%)	20 (41%)	23 (48%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Phenolphthalein
(continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Integumentary System				
Mammary gland	(50)	(50)	(48)	(49)
Galactocele	22 (44%)	15 (30%)	14 (29%)	17 (35%)
Inflammation, chronic		2 (4%)		
Epithelium, hyperplasia		1 (2%)		1 (2%)
Skin	(50)	(50)	(49)	(50)
Subcutaneous tissue, fibrosis, focal			1 (2%)	
Subcutaneous tissue, inflammation, acute			1 (2%)	
Subcutaneous tissue, inflammation, chronic, focal				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Inflammation, acute		1 (2%)		
Femur, fibrous osteodystrophy	1 (2%)			
Femur, hyperostosis	7 (14%)	2 (4%)	3 (6%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hydrocephalus	1 (2%)	1 (2%)	5 (10%)	
Cerebellum, hemorrhage, focal		1 (2%)		
Cerebrum, hemorrhage, focal		1 (2%)	1 (2%)	1 (2%)
Meninges, cerebrum, hemorrhage				1 (2%)
Pons, hemorrhage, focal		1 (2%)		
Thalamus, cerebrum, necrosis		1 (2%)		
Peripheral nerve	(1)	(2)	(2)	(2)
Sciatic, degeneration	1 (100%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body			1 (2%)	
Hemorrhage	2 (4%)	1 (2%)	8 (16%)	4 (8%)
Infiltration cellular, focal, lymphocyte		1 (2%)		
Infiltration cellular, focal, histiocyte				1 (2%)
Inflammation, chronic active	1 (2%)			
Alveolar epithelium, hyperplasia, focal	4 (8%)	5 (10%)		
Interstitium, inflammation, chronic	6 (12%)	5 (10%)	6 (12%)	6 (12%)
Interstitium, inflammation, chronic active		1 (2%)		
Serosa, inflammation, chronic			1 (2%)	
Nose	(50)	(49)	(50)	(50)
Hemorrhage			1 (2%)	
Inflammation, acute		1 (2%)	1 (2%)	
Inflammation, chronic	5 (10%)	2 (4%)	2 (4%)	4 (8%)
Inflammation, chronic active	1 (2%)	6 (12%)	3 (6%)	1 (2%)
Nasolacrimal duct, inflammation, acute		2 (4%)	2 (4%)	2 (4%)
Nasolacrimal duct, inflammation, chronic	1 (2%)			
Nasolacrimal duct, inflammation, chronic active				1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Phenolphthalein
(continued)

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Respiratory System (continued)				
Nose (continued)	(50)	(49)	(50)	(50)
Olfactory epithelium, cytoplasmic alteration	3 (6%)	6 (12%)	3 (6%)	2 (4%)
Turbinate, congestion	1 (2%)			
Trachea	(50)	(50)	(50)	(49)
Inflammation, acute	1 (2%)			
Inflammation, chronic	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Special Senses System				
Eye	(2)	(1)	(2)	
Atrophy		1 (100%)		
Cornea, inflammation, chronic	1 (50%)		1 (50%)	
Lens, cataract	1 (50%)		2 (100%)	
Retina, atrophy	1 (50%)		2 (100%)	
Zymbal's gland		(1)		(1)
Cyst				1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst		2 (4%)		1 (2%)
Ectopic liver				1 (2%)
Fibrosis, focal	1 (2%)	1 (2%)		
Inflammation, acute	1 (2%)		1 (2%)	
Mineralization, focal	3 (6%)	3 (6%)	5 (10%)	3 (6%)
Nephropathy, chronic	4 (8%)	1 (2%)		2 (4%)
Bilateral, mineralization, focal	7 (14%)	5 (10%)	9 (18%)	5 (10%)
Bilateral, nephropathy, chronic	30 (60%)	44 (88%)	43 (86%)	42 (84%)
Bilateral, pelvis, dilatation	1 (2%)			
Bilateral, pelvis, inflammation, acute	1 (2%)			
Bilateral, pelvis, inflammation, chronic active	1 (2%)			
Bilateral, pelvis, mineralization, focal			1 (2%)	
Bilateral, pelvis, transitional epithelium, hyperplasia	2 (4%)			
Bilateral, renal tubule, accumulation, hyaline droplet				2 (4%)
Bilateral, renal tubule, pigmentation	11 (22%)	9 (18%)	7 (14%)	8 (16%)
Papilla, necrosis	1 (2%)			
Pelvis, dilatation			1 (2%)	
Pelvis, inflammation, acute		2 (4%)		
Pelvis, transitional epithelium, hyperplasia		1 (2%)	1 (2%)	
Renal tubule, degeneration	1 (2%)			
Urinary bladder	(49)	(49)	(49)	(49)
Infiltration cellular, focal, lymphocyte	1 (2%)			
Inflammation, chronic			1 (2%)	

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

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

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	6	13	11	3
Natural deaths	4	4	3	10
Survivors				
Terminal sacrifice	40	33	36	36
Missing				1
Animals examined microscopically	50	50	50	49
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Gallbladder	(50)	(46)	(46)	(41)
Fibrosarcoma, metastatic, mesentery		1 (2%)		
Intestine large, rectum	(50)	(50)	(50)	(48)
Fibrosarcoma, metastatic, mesentery			1 (2%)	
Intestine large, cecum	(50)	(48)	(47)	(45)
Carcinoma		1 (2%)		
Intestine small, duodenum	(49)	(48)	(48)	(46)
Carcinoma			1 (2%)	
Fibrosarcoma, metastatic, mesentery		1 (2%)		
Histiocytic sarcoma		1 (2%)		
Intestine small, jejunum	(49)	(49)	(48)	(46)
Fibrosarcoma, metastatic, mesentery			1 (2%)	
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Intestine small, ileum	(49)	(50)	(48)	(46)
Liver	(50)	(50)	(50)	(49)
Fibrosarcoma, metastatic, mesentery		1 (2%)	1 (2%)	
Hemangiosarcoma	1 (2%)	1 (2%)	2 (4%)	
Hemangiosarcoma, multiple		1 (2%)		
Hepatocellular carcinoma	7 (14%)	9 (18%)	4 (8%)	9 (18%)
Hepatocellular carcinoma, multiple	1 (2%)	1 (2%)	1 (2%)	
Hepatocellular adenoma	10 (20%)	11 (22%)	8 (16%)	9 (18%)
Hepatocellular adenoma, multiple	12 (24%)	1 (2%)		1 (2%)
Hepatocholangiocarcinoma		1 (2%)		
Histiocytic sarcoma	1 (2%)	3 (6%)	10 (20%)	12 (24%)
Ito cell tumor malignant	1 (2%)			
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Mesentery	(9)	(9)	(4)	(3)
Fibrosarcoma		1 (11%)	1 (25%)	
Hemangiosarcoma		2 (22%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (11%)		
Sarcoma, metastatic, uncertain primary site		1 (11%)		
Pancreas	(50)	(50)	(50)	(49)
Fibrosarcoma, metastatic, mesentery		1 (2%)	1 (2%)	
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Histiocytic sarcoma				4 (8%)
Sarcoma, metastatic, uncertain primary site		1 (2%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Alimentary System (continued)				
Salivary glands	(50)	(50)	(50)	(49)
Hemangiosarcoma				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(49)
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma			2 (4%)	
Stomach, glandular	(50)	(50)	(50)	(48)
Histiocytic sarcoma		1 (2%)		
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)
Tongue	(1)	(1)		
Squamous cell carcinoma	1 (100%)			
Tooth	(49)	(50)	(50)	(49)
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Fibrosarcoma, metastatic, mesentery		1 (2%)		
Hemangiosarcoma		1 (2%)		
Histiocytic sarcoma				3 (6%)
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Endocrine System				
Adrenal cortex	(49)	(50)	(49)	(48)
Adenoma		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Capsule, adenoma	1 (2%)	2 (4%)		
Capsule, sarcoma, metastatic, uncertain primary site		1 (2%)		
Adrenal medulla	(49)	(50)	(49)	(48)
Islets, pancreatic	(50)	(50)	(50)	(49)
Adenoma	1 (2%)			
Pituitary gland	(48)	(50)	(49)	(48)
Pars distalis, adenoma		1 (2%)		
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(49)	(50)	(50)	(49)
Follicular cell, adenoma		1 (2%)	2 (4%)	
Follicular cell, carcinoma	1 (2%)			
General Body System				
Tissue NOS		(1)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Genital System				
Epididymis	(50)	(50)	(50)	(49)
Fibrosarcoma, metastatic, mesentery		1 (2%)	1 (2%)	
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Histiocytic sarcoma				1 (2%)
Leiomyoma		1 (2%)		
Preputial gland	(49)	(50)	(49)	(49)
Fibrosarcoma, metastatic, skin			1 (2%)	
Prostate	(50)	(50)	(50)	(49)
Fibrosarcoma, metastatic, mesentery		1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(49)
Fibrosarcoma, metastatic, mesentery		1 (2%)	1 (2%)	
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Testes	(50)	(50)	(50)	(48)
Hemangiosarcoma			1 (2%)	
Interstitial cell, adenoma	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Hemangiosarcoma		1 (2%)	1 (2%)	
Histiocytic sarcoma		1 (2%)	5 (10%)	5 (10%)
Mast cell tumor malignant				1 (2%)
Lymph node	(8)	(5)	(14)	(9)
Iliac, fibrosarcoma, metastatic, mesentery		1 (20%)		
Iliac, histiocytic sarcoma			2 (14%)	1 (11%)
Inguinal, mast cell tumor malignant, metastatic, bone marrow				1 (11%)
Mediastinal, carcinoma, metastatic, kidney	1 (13%)			
Mediastinal, sarcoma, metastatic, uncertain primary site		1 (20%)		
Pancreatic, histiocytic sarcoma				1 (11%)
Renal, histiocytic sarcoma			2 (14%)	
Renal, osteosarcoma, metastatic, uncertain primary site			1 (7%)	
Lymph node, mandibular	(49)	(50)	(49)	(49)
Histiocytic sarcoma		2 (4%)		4 (8%)
Lymph node, mesenteric	(50)	(50)	(48)	(49)
Fibrosarcoma, metastatic, mesentery		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		
Histiocytic sarcoma		2 (4%)	4 (8%)	7 (14%)
Spleen	(50)	(50)	(50)	(49)
Fibrosarcoma, metastatic, mesentery		1 (2%)		
Hemangiosarcoma		5 (10%)	1 (2%)	1 (2%)
Histiocytic sarcoma		2 (4%)	6 (12%)	9 (18%)
Thymus	(43)	(46)	(44)	(42)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Fibrosarcoma, metastatic, mesentery		1 (2%)		
Histiocytic sarcoma				2 (5%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Integumentary System				
Skin	(50)	(50)	(50)	(49)
Subcutaneous tissue, fibrosarcoma	1 (2%)		1 (2%)	
Subcutaneous tissue, hemangiosarcoma	1 (2%)		3 (6%)	
Subcutaneous tissue, histiocytic sarcoma		1 (2%)	1 (2%)	
Subcutaneous tissue, sarcoma, metastatic, uncertain primary site		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(48)
Carcinoma, metastatic, kidney	1 (2%)			
Skeletal muscle	(2)	(1)	(1)	(1)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (50%)			
Carcinoma, metastatic, kidney	1 (50%)			
Fibrosarcoma, metastatic, mesentery			1 (100%)	
Hepatocholangiocarcinoma, metastatic, liver		1 (100%)		
Nervous System				
Brain	(50)	(50)	(50)	(49)
Respiratory System				
Lung	(50)	(50)	(50)	(49)
Alveolar/bronchiolar adenoma	8 (16%)	10 (20%)	4 (8%)	10 (20%)
Alveolar/bronchiolar adenoma, multiple				2 (4%)
Alveolar/bronchiolar carcinoma	3 (6%)	5 (10%)	4 (8%)	4 (8%)
Alveolar/bronchiolar carcinoma, multiple				1 (2%)
Carcinoma, metastatic, harderian gland			1 (2%)	
Carcinoma, metastatic, kidney	1 (2%)			
Fibrosarcoma, metastatic, mesentery		1 (2%)		
Hemangiosarcoma	1 (2%)			
Hepatocellular carcinoma, metastatic, liver	2 (4%)	3 (6%)	1 (2%)	3 (6%)
Histiocytic sarcoma		2 (4%)	4 (8%)	9 (18%)
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Nose	(50)	(50)	(50)	(49)
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Special Senses System				
Ear		(2)		
Harderian gland	(3)	(4)	(4)	(3)
Adenoma	3 (100%)	3 (75%)	2 (50%)	1 (33%)
Carcinoma			2 (50%)	1 (33%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Histiocytic sarcoma		2 (4%)	2 (4%)	3 (6%)
Sarcoma, metastatic, uncertain primary site		1 (2%)		
Squamous cell carcinoma, metastatic, stomach, forestomach				1 (2%)
Renal tubule, adenoma			2 (4%)	
Transitional epithelium, carcinoma	1 (2%)			
Urinary bladder	(50)	(50)	(48)	(49)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(49)
Histiocytic sarcoma	1 (2%)	3 (6%)	11 (22%)	12 (24%)
Lymphoma malignant	6 (12%)	8 (16%)	12 (24%)	8 (16%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	40	40	42	38
Total primary neoplasms	63	71	65	62
Total animals with benign neoplasms	29	24	18	19
Total benign neoplasms	37	31	20	23
Total animals with malignant neoplasms	19	29	35	29
Total malignant neoplasms	26	40	45	39
Total animals with metastatic neoplasms	4	5	5	4
Total metastatic neoplasms	9	34	13	6
Total animals with malignant neoplasms of uncertain primary site		1	1	

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

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

Number of Days on Study	2 3 2	5 3 3	5 5 0	5 8 2	5 9 9	6 1 3	6 6 2	6 7 3	7 2 4	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 4 7	0 3 4	0 2 8	0 2 0	0 3 7	0 0 7	0 1 7	0 4 2	0 1 1	0 2 2	0 0 2	0 0 3	0 0 9	0 1 0	0 1 2	0 1 3	0 1 6	0 1 8	0 1 9	0 2 1	0 2 3	0 2 6	0 2 7	0 2 9	0 3 0
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma														X											
Hepatocellular carcinoma												X													
Hepatocellular carcinoma, multiple										X															
Hepatocellular adenoma				X							X						X				X				
Hepatocellular adenoma, multiple													X	X									X	X	
Histiocytic sarcoma										X															
Ito cell tumor malignant																									
Mesentery					+		+											+				+		+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																		+							
Squamous cell carcinoma																		X							
Tooth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung			X																						
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																					X				
Adrenal medulla	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X							
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+
Pituitary gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars intermedia, adenoma								X																	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Follicular cell, carcinoma							X																		
General Body System																									
None																									

+: 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 Phenolphthalein: 0 ppm (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	03232	03233	03235	03238	03239	04040	04043	04049	04001	04004	04005	04006	04008	04014	04015	04024	04025	04031	04036	04041	04044	04045	04046	04048	04050	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Hepatocellular carcinoma	X								X	X				X				X		X						7
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma					X		X		X			X				X								X		10
Hepatocellular adenoma, multiple		X									X			X	X				X	X		X			X	12
Histiocytic sarcoma																										1
Ito cell tumor malignant																	X									1
Mesentery		+								+					+											9
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell carcinoma																										1
Tooth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Capsule, adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	46
Pituitary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell, carcinoma																										1
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	2 3 2	5 3 3	5 5 0	5 8 2	5 9 9	6 1 3	6 6 2	6 7 3	7 2 4	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 4 7	0 3 4	0 2 8	0 2 0	0 3 7	0 0 7	0 1 7	0 4 2	0 1 1	0 2 2	0 0 2	0 0 3	0 0 9	0 1 0	0 1 2	0 1 3	0 1 6	0 1 8	0 1 9	0 2 1	0 2 3	0 2 6	0 2 7	0 2 9	0 3 0
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis						+																			
Preputial gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node					+	+	+									+				+					+
Mediastinal, carcinoma, metastatic, kidney						X																			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	I	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung			X																						
Integumentary System																									
Mammary gland	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma																									
Subcutaneous tissue, hemangiosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, kidney						X																			
Skeletal muscle			+			+																			
Alveolar/bronchiolar carcinoma, metastatic, lung			X																						
Carcinoma, metastatic, kidney						X																			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve									+																
Spinal cord									+																
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma											X			X								X			
Alveolar/bronchiolar carcinoma			X		X					X															
Carcinoma, metastatic, kidney						X																			
Hemangiosarcoma									X																
Hepatocellular carcinoma, metastatic, liver										X															
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 3 2	0 3 3	0 3 5	0 3 8	0 3 9	0 4 0	0 4 3	0 4 9	0 0 1	0 0 4	0 0 5	0 0 6	0 0 8	0 1 4	0 1 5	0 2 4	0 2 5	0 3 1	0 3 6	0 4 1	0 4 4	0 4 5	0 4 6	0 4 8	0 5 0	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma						X																				1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+								+																8
Mediastinal, carcinoma, metastatic, kidney																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	I	+	+	+	+	+	+	+	M	43
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																	X									1
Subcutaneous tissue, hemangiosarcoma														X												1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, kidney																										1
Skeletal muscle																										2
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Carcinoma, metastatic, kidney																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X							X	X				X		X											8
Alveolar/bronchiolar carcinoma																										3
Carcinoma, metastatic, kidney																										1
Hemangiosarcoma																										1
Hepatocellular carcinoma, metastatic, liver										X																2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	2	5	5	5	5	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	3	3	5	8	9	1	6	7	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	2	3	0	2	9	3	2	3	4	7	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	3	2	2	3	0	1	4	1	2	0	0	0	1	1	1	1	1	1	2	2	2	2	2	3
	7	4	8	0	7	7	7	2	1	2	2	3	9	0	2	3	6	8	9	1	3	6	7	9	0
Special Senses System																									
Harderian gland														+			+								
Adenoma														X			X								
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, carcinoma						X																			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma										X															
Lymphoma malignant							X								X										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 3 2	0 3 3	0 3 5	0 3 8	0 3 9	0 4 0	0 4 3	0 4 9	0 0 1	0 0 4	0 0 5	0 0 6	0 0 8	0 1 4	0 1 5	0 2 4	0 2 5	0 3 1	0 3 6	0 4 1	0 4 4	0 4 5	0 4 6	0 4 8	0 5 0	Total Tissues/ Tumors
Special Senses System																										
Harderian gland																+										3
Adenoma																X										3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant	X	X			X					X																6

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm

Number of Days on Study	3 4 7	5 4 9	5 6 0	5 6 3	5 8 3	5 9 7	6 0 2	6 3 4	6 3 7	6 5 8	6 7 6	7 0 3	7 0 7	7 0 8	7 1 4	7 2 1	7 2 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 6 6	0 6 8	0 5 3	0 8 5	0 7 3	0 9 4	0 6 5	0 6 4	0 8 1	0 5 4	0 7 2	0 9 3	0 6 9	0 6 3	0 9 1	0 5 7	0 6 2	0 5 1	0 5 2	0 5 5	0 5 6	0 5 8	0 6 0	0 6 7	0 7 0
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	A	+	M	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery													X												
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, duodenum	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery													X												
Histiocytic sarcoma														X											
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery													X												
Hemangiosarcoma												X													
Hemangiosarcoma, multiple																	X								
Hepatocellular carcinoma			X				X				X			X						X					
Hepatocellular carcinoma, multiple													X												
Hepatocellular adenoma				X	X	X				X			X			X		X			X				
Hepatocellular adenoma, multiple																									
Hepatocholangiocarcinoma												X													
Histiocytic sarcoma										X				X			X								
Sarcoma, metastatic, uncertain primary site			X																						
Mesentery	+	+	+									+		+											
Fibrosarcoma													X												
Hemangiosarcoma																									
Hepatocholangiocarcinoma, metastatic, liver												X													
Sarcoma, metastatic, uncertain primary site			X																						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery													X												
Hepatocholangiocarcinoma, metastatic, liver												X													
Sarcoma, metastatic, uncertain primary site			X																						
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma														X											
Tongue																									
Tooth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, uncertain primary site			X																						

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 7 1	0 7 4	0 7 6	0 7 9	0 8 0	0 8 2	0 8 3	0 8 4	0 8 7	0 8 8	0 8 9	0 9 0	0 9 5	0 9 6	0 9 8	0 9 9	1 0 0	0 5 9	0 6 1	0 7 5	0 7 7	0 7 8	0 8 6	0 9 2	0 9 7	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	46
Fibrosarcoma, metastatic, mesentery																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																								X		1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibrosarcoma, metastatic, mesentery																										1
Histiocytic sarcoma																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Hemangiosarcoma																										1
Hemangiosarcoma, multiple																										1
Hepatocellular carcinoma			X						X						X										X	9
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma								X																X	X	11
Hepatocellular adenoma, multiple													X													1
Hepatocholangiocarcinoma																										1
Histiocytic sarcoma																										3
Sarcoma, metastatic, uncertain primary site																										1
Mesentery						+												+			+					9
Fibrosarcoma																										1
Hemangiosarcoma																		X			X					2
Hepatocholangiocarcinoma, metastatic, liver																										1
Sarcoma, metastatic, uncertain primary site																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Hepatocholangiocarcinoma, metastatic, liver																										1
Sarcoma, metastatic, uncertain primary site																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Tongue					+																					1
Tooth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, uncertain primary site																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm (continued)

Number of Days on Study	3 4 7	5 4 9	5 6 0	5 6 3	5 8 3	5 9 7	6 0 2	6 3 4	6 3 7	6 5 8	6 7 6	7 0 3	7 0 7	7 0 8	7 1 4	7 2 1	7 2 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 6 6	0 6 8	0 5 3	0 8 5	0 7 3	0 9 4	0 6 5	0 6 4	0 8 1	0 5 4	0 7 2	0 9 3	0 6 9	0 6 3	0 9 1	0 5 7	0 6 2	0 5 1	0 5 2	0 5 5	0 5 6	0 5 8	0 6 0	0 6 7	0 7 0
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery													X												
Hemangiosarcoma																	X								
Sarcoma, metastatic, uncertain primary site			X																						
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Hepatocholangiocarcinoma, metastatic, liver											X														
Capsule, adenoma								X																	
Capsule, sarcoma, metastatic, uncertain primary site			X																						
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																						X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma				X																					
General Body System																									
Tissue NOS								+																	
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery													X												
Hepatocholangiocarcinoma, metastatic, liver											X														
Leiomyoma						X																			
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery													X												
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery													X												
Hepatocholangiocarcinoma, metastatic, liver											X														
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma												X													
Histiocytic sarcoma																		X							
Lymph node	+		+					+					+										+		
Iliac, fibrosarcoma, metastatic, mesentery													X												
Mediastinal, sarcoma, metastatic, uncertain primary site			X																						

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm (continued)

Number of Days on Study	729	729	729	729	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	00771	00774	00776	00779	00780	00782	00783	00784	00787	00788	00789	00790	00795	00796	00798	00799	00790	00791	00815	00816	00817	00818	00786	00822	00827	00827	Total Tissues/ Tumors
Cardiovascular System																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																											1
Hemangiosarcoma																											1
Sarcoma, metastatic, uncertain primary site																											1
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma										X																	1
Hepatocholangiocarcinoma, metastatic, liver																											1
Capsule, adenoma																			X								2
Capsule, sarcoma, metastatic, uncertain primary site																											1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma																											1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																											1
General Body System																											
Tissue NOS																											1
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																											1
Hepatocholangiocarcinoma, metastatic, liver																											1
Leiomyoma																											1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																											1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																											1
Hepatocholangiocarcinoma, metastatic, liver																											1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																											1
Histiocytic sarcoma																											1
Lymph node																											5
Iliac, fibrosarcoma, metastatic, mesentery																											1
Mediastinal, sarcoma, metastatic, uncertain primary site																											1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm (continued)

Number of Days on Study	3 4 7	5 4 9	5 6 0	5 6 3	5 8 3	5 9 7	6 0 2	6 3 4	6 3 7	6 5 8	6 7 6	7 0 3	7 0 7	7 0 8	7 1 4	7 2 1	7 2 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 6 6	0 6 8	0 5 3	0 8 5	0 7 3	0 9 4	0 6 5	0 6 4	0 8 1	0 5 4	0 7 2	0 9 3	0 6 9	0 6 3	0 9 1	0 5 7	0 6 2	0 5 1	0 5 2	0 5 5	0 5 6	0 5 8	0 6 0	0 6 7	0 7 0
Hematopoietic System (continued)																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma										X				X											
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery													X												
Hepatocholangiocarcinoma, metastatic, liver											X														
Histiocytic sarcoma										X				X											
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery													X												
Hemangiosarcoma												X					X							X	
Histiocytic sarcoma										X				X											
Thymus	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Fibrosarcoma, metastatic, mesentery													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
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, histiocytic sarcoma														X											
Subcutaneous tissue, sarcoma, metastatic, uncertain primary site			X																						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle											+														
Hepatocholangiocarcinoma, metastatic, liver											X														
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma							X				X				X			X	X						
Alveolar/bronchiolar carcinoma									X													X			
Fibrosarcoma, metastatic, mesentery													X												
Hepatocellular carcinoma, metastatic, liver											X														
Histiocytic sarcoma										X				X											
Sarcoma, metastatic, uncertain primary site			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, uncertain primary site			X																						
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear					+			+																	
Harderian gland															+					+				+	
Adenoma															X					X					

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 7 1	0 7 4	0 7 6	0 7 9	0 8 0	0 8 2	0 8 3	0 8 4	0 8 7	0 8 8	0 8 9	0 9 0	0 9 5	0 9 6	0 9 8	0 9 9	1 0 0	0 5 9	0 6 1	0 7 5	0 7 7	0 7 8	0 8 6	0 9 2	0 9 7	Total Tissues/ Tumors
Hematopoietic System (continued)																										
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Hepatocholangiocarcinoma, metastatic, liver																										1
Histiocytic sarcoma																										2
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Hemangiosarcoma																	X		X							5
Histiocytic sarcoma																										2
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Fibrosarcoma, metastatic, mesentery																										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	6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, histiocytic sarcoma																										1
Subcutaneous tissue, sarcoma, metastatic, uncertain primary site																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Hepatocholangiocarcinoma, metastatic, liver																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X				X			X		X											X		10
Alveolar/bronchiolar carcinoma					X		X			X																5
Fibrosarcoma, metastatic, mesentery																										1
Hepatocellular carcinoma, metastatic, liver			X													X										3
Histiocytic sarcoma																										2
Sarcoma, metastatic, uncertain primary site																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, uncertain primary site																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										2
Harderian gland				+																						4
Adenoma				X																						3

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm (continued)

Number of Days on Study	3 4 7	5 4 9	5 6 0	5 6 3	5 8 3	5 9 7	6 0 2	6 3 4	6 3 7	6 5 8	6 7 6	7 0 3	7 0 7	7 0 8	7 1 4	7 2 1	7 2 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 6 6	0 6 8	0 5 3	0 8 5	0 7 3	0 9 4	0 6 5	0 6 4	0 8 1	0 5 4	0 7 2	0 9 3	0 6 9	0 6 3	0 9 1	0 5 7	0 6 2	0 5 1	0 5 2	0 5 5	0 5 6	0 5 8	0 6 0	0 6 7	0 7 0
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma														X			X								
Sarcoma, metastatic, uncertain primary site			X																						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma										X				X			X								
Lymphoma malignant	X				X	X		X							X							X		

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	
Carcass ID Number	00771	00774	00776	00779	00780	00782	00783	00784	00787	00788	00789	00790	00795	00796	00798	00799	00800	00809	00815	00816	00817	00818	00826	00829	00837	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Sarcoma, metastatic, uncertain primary site																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										3
Lymphoma malignant																				X				X		8

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm

Number of Days on Study	4 2 3	4 2 9 5	5 0 0	5 1 5	5 3 5	5 5 6	6 5 7	6 5 9	6 6 5	6 7 3	6 9 6	7 0 0	7 1 3	7 1 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 0 9	1 2 5	1 1 0	1 4 8	1 5 0	1 1 9	1 0 6	1 2 4	1 3 3	1 0 8	1 3 7	1 2 1	1 1 8	1 2 2	1 0 1	1 0 2	1 0 3	1 0 5	1 0 7	1 1 1	1 1 2	1 1 3	1 1 4	1 1 5	1 1 6
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	M	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	M	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery										X														
Intestine large, cecum	+	+	+	+	+	+	+	+	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																		X						
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery										X														
Osteosarcoma, metastatic, uncertain primary site									X															
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery										X														
Hemangiosarcoma																								
Hepatocellular carcinoma			X			X									X			X						
Hepatocellular carcinoma, multiple										X														
Hepatocellular adenoma		X	X											X				X			X			
Histiocytic sarcoma									X			X	X						X			X		
Osteosarcoma, metastatic, uncertain primary site									X															
Mesentery										+														+
Fibrosarcoma										X														
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery										X														
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																								
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma													X									X		
General Body System																								
None																								

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 1 7	1 2 0	1 2 3	1 2 6	1 2 7	1 2 8	1 3 0	1 3 2	1 3 4	1 3 6	1 3 8	1 4 2	1 4 3	1 4 4	1 4 5	1 4 6	1 4 9	1 0 4	1 2 9	1 3 1	1 3 5	1 3 9	1 4 0	1 4 1	1 4 7	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibrosarcoma, metastatic, mesentery																										1
Osteosarcoma, metastatic, uncertain primary site																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Hemangiosarcoma		X											X													2
Hepatocellular carcinoma																										4
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma					X											X				X						8
Histiocytic sarcoma		X						X	X						X			X								10
Osteosarcoma, metastatic, uncertain primary site																										1
Mesentery				+										+												4
Fibrosarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																	X								X	2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										2
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm (continued)

Number of Days on Study	4 2 3	4 9 5	5 0 0	5 1 5	5 3 5	5 5 6	6 5 7	6 5 9	6 6 5	6 7 3	6 9 6	7 0 0	7 1 3	7 1 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 0 9	1 2 5	1 0 0	1 4 8	1 5 0	1 1 9	1 0 6	1 2 4	1 3 3	1 0 8	1 3 7	1 2 1	1 1 8	1 2 2	1 0 1	1 0 2	1 0 3	1 0 5	1 0 7	1 1 1	1 1 2	1 1 3	1 1 4	1 1 5	1 1 6
Genital System																								
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery										X														
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin						X																		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, mesentery										X														
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																								
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																								
Histiocytic sarcoma												X	X						X					
Lymph node									+	+	+	+		+					+					+
Iliac, histiocytic sarcoma												X							X					
Renal, histiocytic sarcoma																								X
Renal, osteosarcoma, metastatic, uncertain primary site									X															
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X				X						X					X
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma														X										
Histiocytic sarcoma									X				X						X					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
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma						X																			
Subcutaneous tissue, hemangiosarcoma				X										X							X				
Subcutaneous tissue, histiocytic sarcoma																									
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle										+															
Fibrosarcoma, metastatic, mesentery										X															
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	
Carcass ID Number	11017	11020	11023	11026	11027	11028	11030	11032	11034	11036	11038	11042	11043	11044	11045	11046	11049	11044	11029	11031	11035	11039	11040	11041	11047	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49
Fibrosarcoma, metastatic, skin																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, mesentery																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma								X																		1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma										X																1
Histiocytic sarcoma															X			X								5
Lymph node	+			+					+				+	+	+									+		14
Iliac, histiocytic sarcoma																										2
Renal, histiocytic sarcoma															X											2
Renal, osteosarcoma, metastatic, uncertain primary site																										1
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Histiocytic sarcoma																										4
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Histiocytic sarcoma								X							X											6
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	44
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	4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, hemangiosarcoma																										3
Subcutaneous tissue, histiocytic sarcoma															X											1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Fibrosarcoma, metastatic, mesentery																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm (continued)

Number of Days on Study	4 2 3	4 9 5	5 0 0	5 1 5	5 3 5	5 5 6	6 5 7	6 5 9	6 6 5	6 7 3	6 9 6	7 0 0	7 1 3	7 1 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 0 9	1 2 5	1 1 0	1 4 8	1 5 0	1 1 9	1 0 6	1 2 4	1 3 3	1 3 8	1 3 7	1 2 1	1 1 8	1 2 2	1 0 1	1 0 2	1 0 3	1 0 5	1 0 7	1 1 1	1 1 2	1 1 3	1 1 4	1 1 5	1 1 6
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma								X												X			X		
Alveolar/bronchiolar carcinoma																			X						
Carcinoma, metastatic, harderian gland																									
Hepatocellular carcinoma, metastatic, liver																			X						
Histiocytic sarcoma																				X					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland																									
Adenoma																									
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Renal tubule, adenoma													X												
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X			X	X						X				X		X
Lymphoma malignant	X		X		X		X	X			X									X		X			

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 1 7	1 2 0	1 2 3	1 2 6	1 2 7	1 2 8	1 3 0	1 3 2	1 3 4	1 3 6	1 3 8	1 4 2	1 4 3	1 4 4	1 4 5	1 4 6	1 4 9	1 0 4	1 2 9	1 3 1	1 3 5	1 3 9	1 4 0	1 4 1	1 4 7	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma												X														4
Alveolar/bronchiolar carcinoma													X							X	X					4
Carcinoma, metastatic, harderian gland														X												1
Hepatocellular carcinoma, metastatic, liver																										1
Histiocytic sarcoma		X													X			X								4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland														+						+	+		+			4
Adenoma																				X	X					2
Carcinoma														X									X			2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma															X											2
Renal tubule, adenoma																						X				2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma		X						X	X						X			X								11
Lymphoma malignant			X	X									X						X							12

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm

Number of Days on Study	0	5	5	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	2	4	8	3	4	4	5	9	0	1	2	2	2	2	2	2	2	2	2	2	2	2
	3	2	9	5	9	5	6	2	9	3	4	0	3	9	9	9	9	9	9	9	9	9	9
Carcass ID Number	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0	5	9	9	8	9	9	9	5	9	6	7	7	5	5	5	5	5	5	6	6	6	6
	0	7	5	2	3	9	8	3	4	0	4	3	7	1	2	3	5	6	8	9	0	1	2	6	6
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	+	+	+	A	+	A	A	+	A	+	+	+	+	+	M	+	M	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma					X		X				X	X													X
Hepatocellular adenoma		X					X					X													
Hepatocellular adenoma, multiple																									
Histiocytic sarcoma			X			X		X	X	X	X		X					X				X		
Mesentery						+																	+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X						X		X											X			
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																				X					
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, stomach, forestomach																									
Tooth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel							+																		
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X			X			X																
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X																
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 7 0	1 7 4	1 7 6	1 8 0	1 8 1	1 8 2	1 8 4	1 8 5	1 8 6	1 8 7	1 8 8	1 8 9	1 9 4	1 9 6	1 6 3	1 6 5	1 6 8	1 6 9	1 7 1	1 7 2	1 7 5	1 7 8	1 7 9	1 8 9	1 9 1	1 9 7	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	41
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma			X																	X	X			X		9
Hepatocellular adenoma			X	X	X	X				X					X											9
Hepatocellular adenoma, multiple																	X									1
Histiocytic sarcoma					X				X						X											12
Mesentery	+																									3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										4
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell carcinoma																									X	1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Squamous cell carcinoma, metastatic, stomach, forestomach																									X	1
Tooth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										3
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal medulla	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm (continued)

Number of Days on Study	0	5	5	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	2	4	8	3	4	4	5	9	0	1	2	2	2	2	2	2	2	2	2	2	2	2	2
	3	2	9	5	9	5	6	2	9	3	4	0	3	9	9	9	9	9	9	9	9	9	9	9
Carcass ID Number	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0	5	9	9	8	9	9	9	5	9	6	7	7	5	5	5	5	5	5	5	6	6	6	6
	0	7	5	2	3	9	8	3	4	0	4	3	7	1	2	3	5	6	8	9	0	1	2	6
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X						X													X		
Mast cell tumor malignant																								
Lymph node		+																	+				+	
Iliac, histiocytic sarcoma																								
Inguinal, mast cell tumor malignant, metastatic, bone marrow																								
Pancreatic, histiocytic sarcoma																								
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X			X			X									X						
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X			X			X		X							X				X		
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																								
Histiocytic sarcoma			X			X		X	X		X							X				X		
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	I	+	+
Histiocytic sarcoma									X		X													
Integumentary System																								
Mammary gland	M	M	M	+	+	M	M	M	M	M	+	M	M	M	M	M	M	+	M	+	M	+	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																			+					
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X			X				X	X							
Alveolar/bronchiolar adenoma, multiple											X									X				
Alveolar/bronchiolar carcinoma												X											X	
Alveolar/bronchiolar carcinoma, multiple																								
Hepatocellular carcinoma, metastatic, liver											X	X												
Histiocytic sarcoma			X			X		X	X	X	X							X				X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Harderian gland																	+							
Adenoma																	X							
Carcinoma																								

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 7 0	1 7 4	1 7 6	1 8 0	1 8 1	1 8 2	1 8 4	1 8 5	1 8 6	1 8 7	1 8 8	1 9 4	1 9 6	1 6 3	1 6 5	1 6 8	1 6 9	1 7 1	1 7 2	1 7 5	1 7 8	1 7 9	1 8 9	1 9 1	1 9 7	Total Tissues/ Tumors
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma									X					X											5
Mast cell tumor malignant																							X		1
Lymph node									+				+	+	+								+		9
Iliac, histiocytic sarcoma														X											1
Inguinal, mast cell tumor malignant, metastatic, bone marrow																							X		1
Pancreatic, histiocytic sarcoma														X											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																									4
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma														X											7
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma								X																	1
Histiocytic sarcoma									X					X											9
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	M	I	42
Histiocytic sarcoma																									2
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	8
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skeletal muscle																									1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma			X										X			X	X	X			X				10
Alveolar/bronchiolar adenoma, multiple																									2
Alveolar/bronchiolar carcinoma							X												X						4
Alveolar/bronchiolar carcinoma, multiple								X																	1
Hepatocellular carcinoma, metastatic, liver																							X		3
Histiocytic sarcoma														X											9
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																									
Harderian gland	+												+												3
Adenoma																									1
Carcinoma													X												1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm (continued)

Number of Days on Study	0	5	5	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	2	4	8	3	4	4	5	9	0	1	2	2	2	2	2	2	2	2	2	2	2	2	2
	3	2	9	5	9	5	6	2	9	3	4	0	3	9	9	9	9	9	9	9	9	9	9	9
Carcass ID Number	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0	5	9	9	8	9	9	9	5	9	6	7	7	5	5	5	5	5	5	5	6	6	6	6
	0	7	5	2	3	9	8	3	4	0	4	3	7	1	2	3	5	6	8	9	0	1	2	6
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma									X		X													
Squamous cell carcinoma, metastatic, stomach, forestomach																								
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X			X		X	X	X	X		X					X				X		
Lymphoma malignant																			X				X	X

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm (continued)

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	730	
Carcass ID Number	17 70	17 74	17 76	18 80	18 81	18 82	18 84	18 85	18 86	18 87	18 88	19 94	19 96	19 97	16 03	16 05	16 08	16 09	17 11	17 12	17 15	17 18	17 19	18 89	19 01	19 17	Total Tissues/ Tumors
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma															X												3
Squamous cell carcinoma, metastatic, stomach, forestomach																										X	1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma					X				X						X												12
Lymphoma malignant	X				X										X			X		X						8

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

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	1/49 (2%)	3/50 (6%)	0/49 (0%)	0/48 (0%)
Adjusted rate[b]	2.5%	8.2%	0.0%	0.0%
Terminal rate[c]	1/40 (3%)	2/33 (6%)	0/35 (0%)	0/35 (0%)
First incidence (days)	729 (T)	634	—[e]	—
Life table test[d]	P=0.168N	P=0.255	P=0.527N	P=0.527N
Logistic regression test[d]	P=0.158N	P=0.308	P=0.527N	P=0.527N
Cochran-Armitage test[d]	P=0.156N			
Fisher exact test[d]		P=0.316	P=0.500N	P=0.505N
Harderian Gland: Adenoma				
Overall rate	3/50 (6%)	3/50 (6%)	2/50 (4%)	1/49 (2%)
Adjusted rate	7.5%	8.7%	5.6%	2.8%
Terminal rate	3/40 (8%)	2/33 (6%)	2/36 (6%)	1/36 (3%)
First incidence (days)	729 (T)	714	729 (T)	729 (T)
Life table test	P=0.215N	P=0.574	P=0.548N	P=0.343N
Logistic regression test	P=0.208N	P=0.613	P=0.548N	P=0.343N
Cochran-Armitage test	P=0.199N			
Fisher exact test		P=0.661N	P=0.500N	P=0.316N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	4/50 (8%)	2/49 (4%)
Adjusted rate	7.5%	8.7%	11.1%	5.6%
Terminal rate	3/40 (8%)	2/33 (6%)	4/36 (11%)	2/36 (6%)
First incidence (days)	729 (T)	714	729 (T)	729 (T)
Life table test	P=0.451N	P=0.574	P=0.442	P=0.548N
Logistic regression test	P=0.444N	P=0.613	P=0.442	P=0.548N
Cochran-Armitage test	P=0.431N			
Fisher exact test		P=0.661N	P=0.500	P=0.510N
Liver: Hepatocellular Adenoma				
Overall rate	22/50 (44%)	12/50 (24%)	8/50 (16%)	10/49 (20%)
Adjusted rate	53.5%	29.3%	19.6%	25.0%
Terminal rate	21/40 (53%)	6/33 (18%)	5/36 (14%)	7/36 (19%)
First incidence (days)	582	563	495	522
Life table test	P=0.017N	P=0.103N	P=0.006N	P=0.022N
Logistic regression test	P=0.010N	P=0.032N	P=0.003N	P=0.011N
Cochran-Armitage test	P=0.010N			
Fisher exact test		P=0.028N	P=0.002N	P=0.010N
Liver: Hepatocellular Carcinoma				
Overall rate	8/50 (16%)	10/50 (20%)	5/50 (10%)	9/49 (18%)
Adjusted rate	19.5%	25.0%	11.8%	22.0%
Terminal rate	7/40 (18%)	5/33 (15%)	2/36 (6%)	5/36 (14%)
First incidence (days)	727	560	500	639
Life table test	P=0.508	P=0.275	P=0.343N	P=0.420
Logistic regression test	P=0.533	P=0.385	P=0.273N	P=0.474
Cochran-Armitage test	P=0.533			
Fisher exact test		P=0.398	P=0.277N	P=0.482

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	27/50 (54%)	20/50 (40%)	11/50 (22%)	16/49 (33%)
Adjusted rate	64.2%	45.3%	25.8%	38.4%
Terminal rate	25/40 (63%)	10/33 (30%)	6/36 (17%)	11/36 (31%)
First incidence (days)	582	560	495	522
Life table test	P=0.033N	P=0.341N	P=0.005N	P=0.060N
Logistic regression test	P=0.016N	P=0.123N	P=0.001N	P=0.026N
Cochran-Armitage test	P=0.016N			
Fisher exact test		P=0.115N	P<0.001N	P=0.026N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	8/50 (16%)	10/50 (20%)	4/50 (8%)	12/49 (24%)
Adjusted rate	20.0%	27.0%	10.5%	30.6%
Terminal rate	8/40 (20%)	7/33 (21%)	3/36 (8%)	9/36 (25%)
First incidence (days)	729 (T)	602	659	699
Life table test	P=0.211	P=0.255	P=0.229N	P=0.162
Logistic regression test	P=0.225	P=0.352	P=0.203N	P=0.185
Cochran-Armitage test	P=0.226			
Fisher exact test		P=0.398	P=0.178N	P=0.212
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	5/50 (10%)	4/50 (8%)	5/49 (10%)
Adjusted rate	6.5%	14.2%	11.1%	13.5%
Terminal rate	0/40 (0%)	4/33 (12%)	4/36 (11%)	4/36 (11%)
First incidence (days)	550	637	729 (T)	720
Life table test	P=0.324	P=0.291	P=0.450	P=0.314
Logistic regression test	P=0.335	P=0.361	P=0.505	P=0.349
Cochran-Armitage test	P=0.337			
Fisher exact test		P=0.357	P=0.500	P=0.346
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	11/50 (22%)	15/50 (30%)	8/50 (16%)	16/49 (33%)
Adjusted rate	25.2%	39.7%	21.3%	40.9%
Terminal rate	8/40 (20%)	11/33 (33%)	7/36 (19%)	13/36 (36%)
First incidence (days)	550	602	659	699
Life table test	P=0.200	P=0.134	P=0.394N	P=0.126
Logistic regression test	P=0.215	P=0.236	P=0.318N	P=0.160
Cochran-Armitage test	P=0.217			
Fisher exact test		P=0.247	P=0.306N	P=0.168
Skin (Subcutaneous Tissue): Hemangiosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/49 (0%)
Adjusted rate	2.5%	0.0%	7.4%	0.0%
Terminal rate	1/40 (3%)	0/33 (0%)	1/36 (3%)	0/36 (0%)
First incidence (days)	729 (T)	—	515	—
Life table test	P=0.515N	P=0.538N	P=0.275	P=0.521N
Logistic regression test	P=0.496N	P=0.538N	P=0.307	P=0.521N
Cochran-Armitage test	P=0.506N			
Fisher exact test		P=0.500N	P=0.309	P=0.505N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Spleen: Hemangiosarcoma				
Overall rate	0/50 (0%)	5/50 (10%)	1/50 (2%)	1/49 (2%)
Adjusted rate	0.0%	14.0%	2.7%	2.8%
Terminal rate	0/40 (0%)	3/33 (9%)	0/36 (0%)	1/36 (3%)
First incidence (days)	—	703	716	729 (T)
Life table test	P=0.500N	P=0.022	P=0.475	P=0.479
Logistic regression test	P=0.484N	P=0.026	P=0.494	P=0.479
Cochran-Armitage test	P=0.482N			
Fisher exact test		P=0.028	P=0.500	P=0.495
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	6/50 (12%)	7/50 (14%)	2/49 (4%)
Adjusted rate	7.3%	16.9%	18.0%	5.6%
Terminal rate	2/40 (5%)	4/33 (12%)	5/36 (14%)	2/36 (6%)
First incidence (days)	724	703	515	729 (T)
Life table test	P=0.380N	P=0.166	P=0.125	P=0.552N
Logistic regression test	P=0.351N	P=0.197	P=0.154	P=0.534N
Cochran-Armitage test	P=0.350N			
Fisher exact test		P=0.243	P=0.159	P=0.510N
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	11/50 (22%)	12/49 (24%)
Adjusted rate	2.4%	7.9%	28.0%	27.5%
Terminal rate	0/40 (0%)	0/33 (0%)	8/36 (22%)	5/36 (14%)
First incidence (days)	727	658	665	549
Life table test	P<0.001	P=0.255	P=0.002	P=0.002
Logistic regression test	P<0.001	P=0.302	P=0.002	P=0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.309	P=0.002	P<0.001
All Organs: Malignant Lymphoma				
Overall rate	6/50 (12%)	8/50 (16%)	12/50 (24%)	8/49 (16%)
Adjusted rate	14.5%	19.1%	27.2%	22.2%
Terminal rate	5/40 (13%)	3/33 (9%)	6/36 (17%)	8/36 (22%)
First incidence (days)	662	347	423	729 (T)
Life table test	P=0.299	P=0.300	P=0.082	P=0.309
Logistic regression test	P=0.324	P=0.396	P=0.101	P=0.353
Cochran-Armitage test	P=0.310			
Fisher exact test		P=0.387	P=0.096	P=0.371
All Organs: Benign Neoplasms				
Overall rate	29/50 (58%)	24/50 (48%)	18/50 (36%)	19/49 (39%)
Adjusted rate	68.9%	55.0%	43.3%	45.9%
Terminal rate	27/40 (68%)	14/33 (42%)	13/36 (36%)	14/36 (39%)
First incidence (days)	582	563	495	522
Life table test	P=0.058N	P=0.521N	P=0.066N	P=0.095N
Logistic regression test	P=0.030N	P=0.234N	P=0.027N	P=0.043N
Cochran-Armitage test	P=0.030N			
Fisher exact test		P=0.212N	P=0.022N	P=0.043N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
All Organs: Malignant Neoplasms				
Overall rate	19/50 (38%)	29/50 (58%)	35/50 (70%)	29/49 (59%)
Adjusted rate	41.2%	61.3%	71.4%	63.0%
Terminal rate	13/40 (33%)	15/33 (45%)	22/36 (61%)	19/36 (53%)
First incidence (days)	550	347	423	549
Life table test	P=0.057	P=0.021	P=0.002	P=0.030
Logistic regression test	P=0.026	P=0.039	P=0.001	P=0.035
Cochran-Armitage test	P=0.031			
Fisher exact test		P=0.036	P=0.001	P=0.028
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	40/50 (80%)	42/50 (84%)	38/49 (78%)
Adjusted rate	83.3%	81.6%	84.0%	80.8%
Terminal rate	32/40 (80%)	24/33 (73%)	28/36 (78%)	27/36 (75%)
First incidence (days)	550	347	423	522
Life table test	P=0.516	P=0.194	P=0.211	P=0.459
Logistic regression test	P=0.461N	P=0.590	P=0.392	P=0.502N
Cochran-Armitage test	P=0.443N			
Fisher exact test		P=0.598N	P=0.398	P=0.479N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, and spleen; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, 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 Histiocytic Sarcoma in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls
Historical Incidence at EG&G Mason Research Institute/TSI Mason Laboratories, Inc.	
1-Amino-2,4-dibromoanthraquinone	0/50
Acetaminophen	1/50
HC Yellow 4	0/50
Methylphenidate Hydrochloride	0/50
Pentaerythritol Tetranitrate	0/49
Turmeric Oleoresin	0/50
Overall Historical Incidence	
Total	6/1,474 (0.4%)
Standard deviation	1.0%
Range	0%-2%

[a] Data as of 12 May 1995

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

Study	Incidence in Controls
Historical Incidence at EG&G Mason Research Institute/TSI Mason Laboratories, Inc.	
1-Amino-2,4-dibromoanthraquinone	10/50
Acetaminophen	7/50
HC Yellow 4	2/50
Methylphenidate Hydrochloride	3/50
Pentaerythritol Tetranitrate	4/49
Turmeric Oleoresin	1/50
Overall Historical Incidence	
Total	130/1,474 (8.8%)
Standard deviation	6.0%
Range	2%-24%

[a] Data as of 12 May 1995; includes data for histocytic, lymphocytic, mixed, unspecified, or undifferentiated cell type lymphomas

TABLE C4c
Historical Incidence of Hepatocellular Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at EG&G Mason Research Institute/TSI Mason Laboratories, Inc.			
1-Amino-2,4-dibromoanthraquinone	10/50	9/50	18/50
Acetaminophen	11/50	7/50	16/50
HC Yellow 4	8/49	5/49	13/49
Methylphenidate Hydrochloride	18/50	10/50	24/50
Pentaerythritol Tetranitrate	9/48	3/48	11/48
Turmeric Oleoresin	25/50	12/50	30/50
Overall Historical Incidence			
Total	413/1,465 (28.2%)	252/1,465 (17.2%)	596/1,465 (40.7%)
Standard deviation	14.2%	7.1%	14.5%
Range	4%-60%	3%-29%	10%-68%

[a] Data as of 12 May 1995

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

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	6	13	11	3
Natural deaths	4	4	3	10
Survivors				
Terminal sacrifice	40	33	36	36
Missing				1
Animals examined microscopically	50	50	50	49
Alimentary System				
Gallbladder	(50)	(46)	(46)	(41)
Metaplasia	1 (2%)			
Intestine large, rectum	(50)	(50)	(50)	(48)
Edema			1 (2%)	
Intestine large, cecum	(50)	(48)	(47)	(45)
Edema		1 (2%)		
Serosa, inflammation, chronic		1 (2%)		
Intestine small, jejunum	(49)	(49)	(48)	(46)
Hemorrhage	1 (2%)			
Necrosis	1 (2%)			
Liver	(50)	(50)	(50)	(49)
Angiectasis		1 (2%)		1 (2%)
Clear cell focus	24 (48%)	6 (12%)	1 (2%)	
Congestion		2 (4%)		
Eosinophilic focus	22 (44%)	6 (12%)	1 (2%)	1 (2%)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation		6 (12%)	6 (12%)	2 (4%)
Inflammation, chronic	4 (8%)	3 (6%)	7 (14%)	5 (10%)
Mineralization			1 (2%)	1 (2%)
Mixed cell focus	6 (12%)	2 (4%)	1 (2%)	
Necrosis	4 (8%)	4 (8%)	4 (8%)	2 (4%)
Vacuolization cytoplasmic	7 (14%)	4 (8%)	2 (4%)	4 (8%)
Bile duct, cyst	1 (2%)			1 (2%)
Bile duct, inflammation, suppurative	1 (2%)			
Oval cell, hyperplasia	4 (8%)	2 (4%)		1 (2%)
Mesentery	(9)	(9)	(4)	(3)
Inflammation, chronic active		1 (11%)		
Fat, necrosis	7 (78%)	3 (33%)	2 (50%)	2 (67%)
Pancreas	(50)	(50)	(50)	(49)
Inflammation, chronic		1 (2%)		
Acinus, atrophy	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Duct, cyst		1 (2%)		
Salivary glands	(50)	(50)	(50)	(49)
Inflammation, chronic				1 (2%)
Necrosis		1 (2%)		
Duct, cyst			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 Phenolphthalein
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(49)
Hyperkeratosis	1 (2%)			
Hyperplasia, squamous	4 (8%)	7 (14%)	8 (16%)	4 (8%)
Inflammation, chronic		2 (4%)	1 (2%)	
Mineralization	1 (2%)			
Ulcer	1 (2%)	3 (6%)	3 (6%)	1 (2%)
Serosa, inflammation, chronic		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(48)
Cyst			1 (2%)	
Erosion		1 (2%)	2 (4%)	5 (10%)
Mineralization	1 (2%)			
Glands, atrophy, focal			1 (2%)	
Glands, cyst	1 (2%)			
Glands, hyperplasia	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Serosa, inflammation, chronic		1 (2%)		
Tooth	(49)	(50)	(50)	(49)
Degeneration	22 (45%)	12 (24%)	4 (8%)	1 (2%)
Inflammation, chronic		1 (2%)		
Inflammation, chronic active		1 (2%)	1 (2%)	2 (4%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(49)
Cardiomyopathy	1 (2%)	1 (2%)	2 (4%)	5 (10%)
Inflammation, chronic			1 (2%)	
Inflammation, chronic active		1 (2%)		
Mineralization		1 (2%)	2 (4%)	1 (2%)
Myocardium, degeneration				1 (2%)
Valve, inflammation				1 (2%)
Endocrine System				
Adrenal cortex	(49)	(50)	(49)	(48)
Hemorrhage				1 (2%)
Hyperplasia	1 (2%)	1 (2%)		
Hypertrophy, focal	16 (33%)	14 (28%)	10 (20%)	11 (23%)
Capsule, hyperplasia	36 (73%)	37 (74%)	34 (69%)	34 (71%)
Capsule, inflammation, chronic		1 (2%)		
Adrenal medulla	(49)	(50)	(49)	(48)
Hyperplasia	1 (2%)		1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(49)
Hyperplasia	45 (90%)	45 (90%)	44 (88%)	41 (84%)
Parathyroid gland	(46)	(48)	(48)	(45)
Cyst			2 (4%)	
Pituitary gland	(48)	(50)	(49)	(48)
Pars distalis, cyst	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Pars distalis, hyperplasia		1 (2%)	1 (2%)	
Thyroid gland	(49)	(50)	(50)	(49)
Cyst				1 (2%)
Follicular cell, hyperplasia	5 (10%)	4 (8%)		1 (2%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Phenolphthalein
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(49)
Granuloma sperm				1 (2%)
Inflammation, chronic		1 (2%)		
Inflammation, granulomatous		1 (2%)		
Spermatocele	1 (2%)	1 (2%)		
Penis	(1)			
Hemorrhage	1 (100%)			
Preputial gland	(49)	(50)	(49)	(49)
Cyst	39 (80%)	37 (74%)	41 (84%)	47 (96%)
Granuloma			1 (2%)	
Hyperplasia, lymphoid			1 (2%)	
Inflammation, acute		1 (2%)		
Inflammation, chronic	5 (10%)	1 (2%)	3 (6%)	3 (6%)
Inflammation, chronic active	1 (2%)	2 (4%)	5 (10%)	7 (14%)
Prostate	(50)	(50)	(50)	(49)
Cyst		1 (2%)		
Inflammation, chronic		1 (2%)		
Seminal vesicle	(50)	(50)	(50)	(49)
Atrophy				1 (2%)
Hypertrophy	1 (2%)			
Testes	(50)	(50)	(50)	(48)
Germinal epithelium, degeneration	1 (2%)	49 (98%)	50 (100%)	47 (98%)
Interstitial cell, hyperplasia		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Angiectasis			2 (4%)	
Myelofibrosis	3 (6%)	8 (16%)	8 (16%)	19 (39%)
Pigmentation		2 (4%)	5 (10%)	16 (33%)
Thrombosis		2 (4%)		
Myeloid cell, hyperplasia		1 (2%)		
Lymph node	(8)	(5)	(14)	(9)
Iliac, angiectasis			1 (7%)	
Iliac, hyperplasia				1 (11%)
Iliac, hyperplasia, lymphoid	1 (13%)		2 (14%)	
Iliac, pigmentation	1 (13%)			
Inguinal, hyperplasia, lymphoid	3 (38%)		2 (14%)	1 (11%)
Inguinal, pigmentation			1 (7%)	
Mediastinal, hyperplasia, lymphoid	1 (13%)		1 (7%)	
Mediastinal, infiltration cellular, plasma cell				1 (11%)
Pancreatic, angiectasis	1 (13%)		1 (7%)	
Renal, angiectasis			1 (7%)	
Renal, hyperplasia, lymphoid				1 (11%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Phenolphthalein
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Hematopoietic System (continued)				
Lymph node, mandibular	(49)	(50)	(49)	(49)
Angiectasis				1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Infiltration cellular, histiocyte				1 (2%)
Lymph node, mesenteric	(50)	(50)	(48)	(49)
Angiectasis	11 (22%)	10 (20%)	13 (27%)	12 (24%)
Hyperplasia, lymphoid	3 (6%)	2 (4%)		3 (6%)
Infiltration cellular, polymorphonuclear	2 (4%)			
Infiltration cellular, histiocyte			1 (2%)	
Inflammation, chronic		1 (2%)		
Spleen	(50)	(50)	(50)	(49)
Angiectasis		1 (2%)		
Hematopoietic cell proliferation	10 (20%)	22 (44%)	28 (56%)	21 (43%)
Pigmentation	3 (6%)	5 (10%)		4 (8%)
Lymphoid follicle, atrophy	1 (2%)			
Lymphoid follicle, hyperplasia	2 (4%)	2 (4%)	4 (8%)	
Lymphoid follicle, necrosis	1 (2%)			
Thymus	(43)	(46)	(44)	(42)
Atrophy	13 (30%)	11 (24%)	16 (36%)	12 (29%)
Cyst		1 (2%)		
Hyperplasia, atypical		3 (7%)	7 (16%)	7 (17%)
Hyperplasia, oncocytic	1 (2%)			
Necrosis	1 (2%)			
Integumentary System				
Skin	(50)	(50)	(50)	(49)
Inflammation, acute			1 (2%)	1 (2%)
Inflammation, chronic		2 (4%)		1 (2%)
Subcutaneous tissue, edema		2 (4%)	7 (14%)	6 (12%)
Subcutaneous tissue, infiltration cellular, polymorphonuclear				1 (2%)
Subcutaneous tissue, inflammation, acute			1 (2%)	1 (2%)
Subcutaneous tissue, inflammation, chronic				1 (2%)
Subcutaneous tissue, necrosis		1 (2%)		
Musculoskeletal System				
None				
Nervous System				
Brain	(50)	(50)	(50)	(49)
Hemorrhage		1 (2%)		1 (2%)
Necrosis	1 (2%)			
Thalamus, mineralization	27 (54%)	24 (48%)	25 (50%)	35 (71%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of Phenolphthalein
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Respiratory System				
Lung	(50)	(50)	(50)	(49)
Congestion	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Hemorrhage	1 (2%)	3 (6%)	4 (8%)	7 (14%)
Infiltration cellular, histiocyte	5 (10%)	5 (10%)	2 (4%)	
Inflammation, granulomatous	1 (2%)			
Alveolar epithelium, hyperplasia	3 (6%)	5 (10%)	3 (6%)	3 (6%)
Nose	(50)	(50)	(50)	(49)
Inflammation, acute	2 (4%)			
Polyp, inflammatory	1 (2%)	1 (2%)		
Special Senses System				
Harderian gland	(3)	(4)	(4)	(3)
Hypertrophy		1 (25%)		2 (67%)
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Cyst	7 (14%)	5 (10%)	5 (10%)	4 (8%)
Hydronephrosis	2 (4%)			
Infarct				1 (2%)
Infarct, chronic				1 (2%)
Inflammation, acute				1 (2%)
Metaplasia, osseous	1 (2%)			
Mineralization	7 (14%)	1 (2%)	2 (4%)	
Nephropathy, chronic	47 (94%)	46 (92%)	42 (84%)	41 (84%)
Pigmentation			1 (2%)	
Papilla, necrosis	1 (2%)			
Pelvis, angiectasis	1 (2%)			
Renal tubule, accumulation, hyaline droplet		2 (4%)	5 (10%)	5 (10%)
Renal tubule, dilatation	1 (2%)		1 (2%)	2 (4%)
Renal tubule, hyperplasia	1 (2%)			
Renal tubule, necrosis				1 (2%)
Transitional epithelium, hyperplasia			1 (2%)	
Urinary bladder	(50)	(50)	(48)	(49)
Hemorrhage		1 (2%)		
Inflammation, acute				1 (2%)
Serosa, inflammation, chronic		1 (2%)		

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

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

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	13	9	12
Natural deaths	6	6	7	10
Survivors				
Died last week of the study	1			
Terminal sacrifice	38	31	34	28
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(45)	(46)	(46)	(44)
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)
Intestine large, colon	(50)	(48)	(48)	(48)
Intestine large, rectum	(50)	(49)	(48)	(49)
Intestine large, cecum	(50)	(48)	(48)	(45)
Fibrosarcoma		1 (2%)		
Intestine small, duodenum	(48)	(47)	(47)	(48)
Intestine small, jejunum	(49)	(48)	(46)	(46)
Carcinoma			1 (2%)	
Intestine small, ileum	(48)	(47)	(46)	(47)
Liver	(50)	(50)	(50)	(50)
Fibrous histiocytoma	1 (2%)			
Hemangiosarcoma, metastatic, spleen		1 (2%)		
Hepatocellular carcinoma	6 (12%)	1 (2%)		1 (2%)
Hepatocellular adenoma	12 (24%)	2 (4%)	6 (12%)	1 (2%)
Hepatocellular adenoma, multiple	5 (10%)			
Histiocytic sarcoma		1 (2%)	6 (12%)	7 (14%)
Mesentery	(16)	(2)	(7)	(1)
Fibrous histiocytoma	1 (6%)			
Hemangiosarcoma			1 (14%)	
Pancreas	(50)	(50)	(50)	(50)
Fibrous histiocytoma	1 (2%)			
Histiocytic sarcoma			1 (2%)	1 (2%)
Salivary glands	(49)	(50)	(50)	(49)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma		1 (2%)		
Squamous cell papilloma	1 (2%)	1 (2%)		3 (6%)
Stomach, glandular	(50)	(49)	(49)	(50)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			1 (2%)
Fibrous histiocytoma	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma			1 (2%)	
Pituitary gland	(49)	(50)	(49)	(48)
Pars distalis, adenoma	3 (6%)	2 (4%)	1 (2%)	
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	1 (2%)	2 (4%)		
General Body System				
Tissue NOS		(1)		
Genital System				
Clitoral gland	(48)	(48)	(48)	(50)
Ovary	(50)	(49)	(50)	(50)
Cystadenoma		3 (6%)		1 (2%)
Fibrous histiocytoma	1 (2%)			
Granulosa cell tumor benign	1 (2%)	1 (2%)		
Hemangioma	2 (4%)			1 (2%)
Histiocytic sarcoma		1 (2%)		
Sex-cord stromal tumor, benign		6 (12%)	6 (12%)	5 (10%)
Teratoma benign	1 (2%)			
Bilateral, sex-cord stromal tumor, benign		1 (2%)		
Uterus	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)			
Histiocytic sarcoma		1 (2%)		
Polyp stromal	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangioma			1 (2%)	
Histiocytic sarcoma		1 (2%)	2 (4%)	5 (10%)
Lymph node	(14)	(17)	(13)	(16)
Iliac, fibrous histiocytoma	1 (7%)			
Iliac, histiocytic sarcoma				3 (19%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung	2 (14%)			
Mediastinal, histiocytic sarcoma				1 (6%)
Pancreatic, histiocytic sarcoma				1 (6%)
Renal, histiocytic sarcoma				2 (13%)
Lymph node, mandibular	(49)	(50)	(49)	(49)
Histiocytic sarcoma			3 (6%)	4 (8%)
Lymph node, mesenteric	(49)	(50)	(49)	(50)
Fibrous histiocytoma	1 (2%)		1 (2%)	
Histiocytic sarcoma		1 (2%)	3 (6%)	4 (8%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Hematopoietic System (continued)				
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)	2 (4%)	1 (2%)
Histiocytic sarcoma		1 (2%)	3 (6%)	6 (12%)
Thymus	(48)	(44)	(49)	(45)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Fibrous histiocytoma	1 (2%)			
Histiocytic sarcoma			1 (2%)	2 (4%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoacanthoma			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)		
Trichoepithelioma			1 (2%)	
Subcutaneous tissue, hemangiosarcoma			1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma		1 (2%)		1 (2%)
Skeletal muscle	(1)	(1)	(1)	
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (100%)			
Hemangiosarcoma		1 (100%)	1 (100%)	
Nervous System				
Brain	(50)	(49)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	1 (2%)	4 (8%)	6 (12%)
Alveolar/bronchiolar adenoma, multiple			1 (2%)	
Alveolar/bronchiolar carcinoma	4 (8%)	4 (8%)	1 (2%)	2 (4%)
Carcinoma, metastatic, uncertain primary site		1 (2%)		
Fibrous histiocytoma	1 (2%)			
Hepatocellular carcinoma, metastatic, liver	2 (4%)	1 (2%)		
Histiocytic sarcoma		1 (2%)	4 (8%)	4 (8%)
Osteosarcoma, metastatic, bone				1 (2%)
Nose	(50)	(50)	(50)	(50)
Carcinoma, metastatic, harderian gland				1 (2%)
Hemangiosarcoma			1 (2%)	
Osteosarcoma, metastatic, bone		1 (2%)		
Trachea	(50)	(50)	(50)	(50)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Special Senses System				
Harderian gland	(5)	(2)	(1)	(2)
Adenoma	2 (40%)	1 (50%)	1 (100%)	1 (50%)
Carcinoma	2 (40%)			1 (50%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Histiocytic sarcoma				1 (2%)
Urinary bladder	(50)	(50)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		2 (4%)	7 (14%)	7 (14%)
Lymphoma malignant	15 (30%)	28 (56%)	33 (66%)	25 (50%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	40	39	47	43
Total primary neoplasms	70	63	75	58
Total animals with benign neoplasms	26	20	23	17
Total benign neoplasms	33	23	25	19
Total animals with malignant neoplasms	25	34	41	37
Total malignant neoplasms	37	40	50	39
Total animals with metastatic neoplasms	3	4		3
Total metastatic neoplasms	7	4		4
Total animals with malignant neoplasms of uncertain primary site		1		

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

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

Number of Days on Study	0 7 8	1 5 6	5 4 6	5 9 7	6 1 3	6 1 5	6 2 8	6 8 6	7 0 8	7 2 3	7 3 0	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7
Carcass ID Number	2 4 3	2 5 7	2 5 2	2 4 5	2 5 8	2 5 6	2 5 3	2 2 2	2 5 1	2 2 6	2 3 7	2 1 8	2 2 0	2 2 1	2 2 3	2 2 4	2 2 7	2 2 8	2 2 9	2 3 1	2 3 2	2 3 3	2 3 6	2 3 8	2 3 9
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	A	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																									
Hepatocellular carcinoma									X														X		X
Hepatocellular adenoma				X											X	X	X		X						
Hepatocellular adenoma, multiple								X						X											X
Mesentery							+								+	+		+						+	
Fibrous histiocytoma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																								X	
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung									X																
Fibrous histiocytoma																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Pars distalis, adenoma																									
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									X
General Body System																									
None																									

+: 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 Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	2 4 0	2 4 1	2 4 2	2 4 8	2 4 9	2 5 4	2 5 5	2 6 1	2 6 2	2 6 3	2 6 5	2 6 6	2 1 7	2 1 9	2 2 5	2 3 0	2 3 4	2 3 5	2 4 4	2 4 6	2 4 7	2 5 0	2 5 9	2 6 0	2 6 4	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma					X																					1
Hepatocellular carcinoma		X																X							X	6
Hepatocellular adenoma		X	X				X							X		X	X						X			12
Hepatocellular adenoma, multiple					X														X							5
Mesentery	+		+		+	+					+		+	+		+				+		+		+		16
Fibrous histiocytoma					X																					1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma					X																					1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Fibrous histiocytoma					X																					1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma			X											X						X						3
Pars intermedia, adenoma						X																				1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	0 7 8	1 5 6	5 4 6	5 9 7	6 1 3	6 1 5	6 2 8	6 8 6	7 0 8	7 2 3	7 3 0	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7
Carcass ID Number	2 4 3	2 5 7	2 5 2	2 4 5	2 5 8	2 5 6	2 5 3	2 2 2	2 5 1	2 2 6	2 3 7	2 1 8	2 2 0	2 2 1	2 2 3	2 2 4	2 2 7	2 2 8	2 2 9	2 3 1	2 3 2	2 3 3	2 3 6	2 3 8	2 3 9
Genital System																									
Clitoral gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																									
Granulosa cell tumor benign																			X						
Hemangioma								X																	
Teratoma benign	X																								
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Polyp stromal																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+	+		+	+	+							+						+		+	
Iliac, fibrous histiocytoma																									
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung				X					X																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle				+																					
Alveolar/bronchiolar carcinoma, metastatic, lung				X																					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve			+																						
Spinal cord			+																						
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X						X													
Alveolar/bronchiolar carcinoma				X					X																
Fibrous histiocytoma																									
Hepatocellular carcinoma, metastatic, liver									X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	2 4 0	2 4 1	2 4 2	2 4 8	2 4 9	2 5 4	2 5 5	2 6 1	2 6 2	2 6 3	2 6 5	2 6 6	2 1 7	2 1 7	2 1 9	2 2 5	2 3 0	2 3 4	2 3 5	2 4 4	2 4 6	2 4 7	2 5 0	2 5 9	2 6 0	2 6 4	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma					X																					1
Granulosa cell tumor benign																										1
Hemangioma																							X			2
Teratoma benign																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																								X		1
Polyp stromal							X																			1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node					+	+									+		+		+		+					14
Iliac, fibrous histiocytoma					X																					1
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrous histiocytoma					X																					1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibrous histiocytoma					X																					1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																				X						3
Alveolar/bronchiolar carcinoma									X											X						4
Fibrous histiocytoma					X																					1
Hepatocellular carcinoma, metastatic, liver																									X	2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	0	1	5	5	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	7	5	4	9	1	1	2	8	0	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	8	6	6	7	3	5	8	6	8	3	0	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Carcass ID Number	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	4	5	5	4	5	5	5	2	5	2	3	1	2	2	2	2	2	2	2	3	3	3	3	3	3
	3	7	2	5	8	6	3	2	1	6	7	8	0	1	3	4	7	8	9	1	2	3	6	8	9
Special Senses System																									
Ear										+															
Harderian gland											+	+						+							
Adenoma											X														
Carcinoma												X						X							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung									X																
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant		X			X	X	X							X		X			X			X			

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 0 ppm (continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	2 4 0	2 4 1	2 4 2	2 4 8	2 4 9	2 5 4	2 5 5	2 6 1	2 6 2	2 6 3	2 6 5	2 6 6	2 1 6	2 1 7	2 1 9	2 2 5	2 3 0	2 3 4	2 3 5	2 4 4	2 4 6	2 4 7	2 5 0	2 5 9	2 6 0	2 6 4	Total Tissues/ Tumors
Special Senses System																										
Ear																										1
Harderian gland				+	+																					5
Adenoma				X																						2
Carcinoma																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant		X								X						X			X		X	X			X	15

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm

Number of Days on Study	1 2 4 4 5 5 5 5 5 5 5 6 6 6 6 6 7 7 7 7 7 7 7 7 2 6 3 5 0 1 5 7 7 8 8 0 1 4 5 6 6 0 0 3 3 3 3 3 3 6 6 5 0 0 1 0 0 8 1 1 8 5 3 9 3 8 3 8 7 7 7 7 7 7
Carcass ID Number	3 2 3 2 2 2 2 2 2 2 2 2 2 2 3 3 2 3 2 2 2 2 2 2 0 6 0 8 8 8 7 7 6 8 9 9 8 7 0 0 9 0 9 6 6 7 7 7 9 6 1 2 8 0 2 1 9 9 0 2 6 7 0 2 4 7 7 7 8 0 3 4 8
Alimentary System	
Esophagus	+ +
Gallbladder	+ A + + + + + + + + + + A + + A + + + + + + + +
Histiocytic sarcoma	X
Intestine large, colon	+ + + + + + + + + + A + A + + + + + + + + + + +
Intestine large, rectum	+ + + + + + + + + + A + + + + + + + + + + + +
Intestine large, cecum	+ + + + + + + + + + A + A + + + + + + + + + + +
Fibrosarcoma	
Intestine small, duodenum	+ A + + + + + + + + A + A + + + + + + + + + + +
Intestine small, jejunum	+ + + + + + + + + + A + A + + + + + + + + + + +
Intestine small, ileum	+ A + + + + + + + + A + A + + + + + + + + + + +
Liver	+ +
Hemangiosarcoma, metastatic, spleen	
Hepatocellular carcinoma	
Hepatocellular adenoma	
Histiocytic sarcoma	X
Mesentery	+
Pancreas	+ +
Salivary glands	+ +
Stomach, forestomach	+ +
Squamous cell carcinoma	
Squamous cell papilloma	
Stomach, glandular	+ + + + + + + + + + A + + + + + + + + + + + + +
Cardiovascular System	
Heart	+ +
Endocrine System	
Adrenal cortex	+ +
Adrenal medulla	+ +
Islets, pancreatic	+ +
Parathyroid gland	+ + + + + + + + + + + + + + M + + + + + + + M + +
Pituitary gland	+ +
Pars distalis, adenoma	
Thyroid gland	+ +
Follicular cell, adenoma	X X
General Body System	
Tissue NOS	+
Genital System	
Clitoral gland	M +
Ovary	+ + + + + + + + + + + + + + + I + + + + + + + + +
Cystadenoma	X
Granulosa cell tumor benign	
Histiocytic sarcoma	X
Sex-cord stromal tumor, benign	X X X
Bilateral, sex-cord stromal tumor, benign	
Uterus	+ +
Histiocytic sarcoma	X
Polyp stromal	X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm (continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	2 7 9	2 8 1	2 8 4	2 8 5	2 8 7	2 9 1	2 9 3	2 9 5	2 9 6	2 9 9	3 0 4	3 0 5	3 0 8	3 1 1	3 1 3	3 1 4	3 1 5	2 7 5	2 7 6	2 8 3	2 8 8	3 0 3	3 0 6	3 1 0	3 1 2	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	46
Histiocytic sarcoma																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibrosarcoma																						X				1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, spleen											X															1
Hepatocellular carcinoma								X																		1
Hepatocellular adenoma				X																			X			2
Histiocytic sarcoma																										1
Mesentery																									+	2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																X										1
Squamous cell papilloma							X																			1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma					X				X																	2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										2
General Body System																										
Tissue NOS																										1
Genital System																										
Clitoral gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cystadenoma															X	X										3
Granulosa cell tumor benign									X																	1
Histiocytic sarcoma																										1
Sex-cord stromal tumor, benign						X														X		X				6
Bilateral, sex-cord stromal tumor, benign																		X								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Polyp stromal																			X							2

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm (continued)

Number of Days on Study	1 2 4 4 5 5 5 5 5 5 6 6 6 6 6 7 7 7 7 7 7 7 7 2 6 3 5 0 1 5 7 7 8 8 0 1 4 5 6 6 0 0 3 3 3 3 3 3 6 6 5 0 0 1 0 0 8 1 1 8 5 3 9 3 8 3 8 7 7 7 7 7 7
Carcass ID Number	3 2 3 2 2 2 2 2 2 2 2 2 2 2 3 3 2 3 2 2 2 2 2 2 0 6 0 8 8 8 7 7 6 8 9 9 8 7 0 0 9 0 9 6 6 7 7 7 9 6 1 2 8 0 2 1 9 9 0 2 6 7 0 2 4 7 7 7 8 0 3 4 8
Hematopoietic System	
Bone marrow	+ +
Histiocytic sarcoma	X
Lymph node	+ +
Lymph node, mandibular	+ +
Lymph node, mesenteric	+ +
Histiocytic sarcoma	X
Spleen	+ +
Hemangiosarcoma	
Histiocytic sarcoma	X
Thymus	+ + + + + + + + + + + M + + M M + + + + + + + + +
Integumentary System	
Mammary gland	+ +
Skin	+ +
Basal cell adenoma	
Musculoskeletal System	
Bone	+ +
Osteosarcoma	X
Skeletal muscle	
Hemangiosarcoma	
Nervous System	
Brain	+ + + + + + + + + + + + + + + + + M + + + + + + +
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	
Alveolar/bronchiolar carcinoma	X
Carcinoma, metastatic, uncertain primary site	X
Hepatocellular carcinoma, metastatic, liver	
Histiocytic sarcoma	X
Nose	+ +
Osteosarcoma, metastatic, bone	X
Trachea	+ +
Special Senses System	
Ear	+ +
Harderian gland	+
Adenoma	
Urinary System	
Kidney	+ +
Urinary bladder	+ +
Systemic Lesions	
Multiple organs	+ +
Histiocytic sarcoma	X X
Lymphoma malignant	X X X X X X X X X X X X X X X X X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 3,000 ppm (continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	2 7 9	2 8 1	2 8 4	2 8 5	2 8 7	2 9 1	2 9 3	2 9 5	2 9 6	2 9 9	3 0 4	3 0 5	3 0 8	3 1 1	3 1 3	3 1 4	3 1 5	2 7 5	2 7 6	2 8 3	2 8 8	3 0 3	3 0 6	3 1 0	3 1 2	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node				+						+	+															17
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma											X															1
Histiocytic sarcoma																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	I	+	+	+	+	+	+	+	M	44
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma						X																				1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Skeletal muscle											+															1
Hemangiosarcoma											X															1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma											X															1
Alveolar/bronchiolar carcinoma															X			X				X				4
Carcinoma, metastatic, uncertain primary site																										1
Hepatocellular carcinoma, metastatic, liver								X																		1
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, bone																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										2
Harderian gland																+										2
Adenoma																X										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										2
Lymphoma malignant				X	X					X	X		X		X	X						X	X			28

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm

Number of Days on Study	3 2 9	3 3 8	4 7 9	5 5 1	5 6 1	5 6 7	6 0 2	6 0 4	6 1 5	6 2 2	6 4 5	6 5 1	6 5 4	6 8 6	6 8 6	6 9 3	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7
Carcass ID Number	3 5 8	3 3 7	3 6 2	3 6 4	3 2 1	3 4 6	3 3 2	3 1 6	3 2 5	3 4 0	3 6 3	3 4 5	3 5 7	3 2 9	3 3 8	3 4 1	3 1 8	3 1 9	3 2 0	3 2 2	3 2 4	3 2 7	3 3 8	3 3 0	3 3 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	A	+	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma											X														
Intestine large, colon	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	A	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, ileum	A	+	+	A	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																X					X				
Histiocytic sarcoma											X	X			X										
Mesentery						+					+								+						
Hemangiosarcoma						X																			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																						X			
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																				X					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sex-cord stromal tumor, benign																							X		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																X									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm (continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	3 3 3	3 3 5	3 3 6	3 3 9	3 4 2	3 4 3	3 4 4	3 4 7	3 4 8	3 4 9	3 5 1	3 5 4	3 5 5	3 5 6	3 5 9	3 6 0	3 6 1	3 6 5	3 7 7	3 1 3	3 2 6	3 2 4	3 3 0	3 5 2	3 5 3	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Histiocytic sarcoma																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Carcinoma												X														1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma		X							X					X							X					6
Histiocytic sarcoma					X				X			X														6
Mesentery		+			+												+			+						7
Hemangiosarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma					X																					1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth																	+									1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sex-cord stromal tumor, benign				X											X			X			X			X		6
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal					X						X															3

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm (continued)

Number of Days on Study	3 2 9	3 3 8	4 7 9	5 5 1	5 6 1	5 6 7	6 0 2	6 0 4	6 1 5	6 2 2	6 4 5	6 5 1	6 5 4	6 8 6	6 8 6	6 9 3	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7
Carcass ID Number	3 5 8	3 3 7	3 6 2	3 6 4	3 2 1	3 4 6	3 3 2	3 1 6	3 2 5	3 4 0	3 6 3	3 4 5	3 5 7	3 2 9	3 3 8	3 4 1	3 1 8	3 1 9	3 2 0	3 2 2	3 2 4	3 2 7	3 2 8	3 3 0	3 3 1
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma						X																			
Histiocytic sarcoma											X														
Lymph node		+			+				+	+			+	+				+						+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Histiocytic sarcoma											X	X													
Lymph node, mesenteric	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma																		X							
Histiocytic sarcoma											X	X													
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma						X																			
Histiocytic sarcoma												X			X										
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma						X																			
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoacanthoma																X									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trichoepithelioma			X																						
Subcutaneous tissue, hemangiosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Hemangiosarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve					+																				
Spinal cord					+																				
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								X	X
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma																X									
Histiocytic sarcoma											X	X			X										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland																									
Adenoma																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm (continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	3 3 3	3 3 5	3 3 6	3 3 9	3 4 2	3 4 3	3 4 4	3 4 7	3 4 8	3 4 9	3 5 1	3 5 4	3 5 5	3 5 6	3 5 9	3 6 0	3 6 1	3 6 5	3 1 7	3 2 3	3 2 6	3 3 4	3 5 0	3 5 2	3 5 3	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Histiocytic sarcoma					X																					2
Lymph node	+	+		+																			+		+	13
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma					X																					3
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrous histiocytoma																										1
Histiocytic sarcoma					X																					3
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma														X												2
Histiocytic sarcoma									X																	3
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoacanthoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trichoepithelioma																										1
Subcutaneous tissue, hemangiosarcoma														X												1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																+										1
Hemangiosarcoma																X										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X						X																4
Alveolar/bronchiolar adenoma, multiple														X												1
Alveolar/bronchiolar carcinoma																										1
Histiocytic sarcoma					X																					4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																				X						1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland							+																			1
Adenoma							X																			1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm (continued)

Number of Days on Study	3	3	4	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	
	2	3	7	5	6	6	0	0	1	2	4	5	5	8	8	9	3	3	3	3	3	3	3	3	3	
	9	8	9	1	1	7	2	4	5	2	5	1	4	6	6	3	7	7	7	7	7	7	7	7	7	
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	5	3	6	6	2	4	3	1	2	4	6	4	5	2	3	4	1	1	2	2	2	2	2	3	3	
	8	7	2	4	1	6	2	6	5	0	3	5	7	9	8	1	8	9	0	2	4	7	8	0	1	
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Histiocytic sarcoma						X					X	X			X											
Lymphoma malignant		X		X	X		X	X	X	X			X	X	X		X	X	X		X	X	X	X	X	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 6,000 ppm (continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	3 3 3	3 3 5	3 3 6	3 3 9	3 4 2	3 4 3	3 4 4	3 4 7	3 4 8	3 4 9	3 5 1	3 5 4	3 5 5	3 5 6	3 5 9	3 6 0	3 6 1	3 6 5	3 1 7	3 2 3	3 2 6	3 3 4	3 5 0	3 5 2	3 5 3	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma					X				X			X														7
Lymphoma malignant	X	X	X	X		X					X			X	X		X	X	X	X	X		X		X	33

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm

Number of Days on Study	0 4 4 4 5 5 5 5 5 5 5 5 6 6 6 6 6 7 7 7 7 7 7 4 4 4 5 0 1 6 7 7 8 8 9 9 0 0 4 5 6 8 1 2 3 3 3 3 0 7 9 0 5 4 8 3 8 8 9 4 8 1 2 0 2 7 0 5 2 0 7 7 7
Carcass ID Number	4 3 3 3 4 4 3 3 3 3 3 3 3 3 4 3 3 4 3 4 3 4 3 3 3 1 9 8 9 1 1 9 9 8 8 9 9 8 7 0 8 6 1 8 0 6 0 6 6 7 4 8 0 9 1 0 4 7 8 3 2 0 9 8 1 7 9 3 6 6 8 7 6 7 0
Alimentary System	
Esophagus	+ +
Gallbladder	+ + + + + + + A + A A + A A + + A + + + + + + + +
Histiocytic sarcoma	X
Intestine large, colon	+ + + + + + + + + + + + A A + + + + + + + + + +
Intestine large, rectum	+ + + + + + + + + + + + + A + + + + + + + + + +
Intestine large, cecum	+ + + + + + + + + A + + A A + + A + + + + A + +
Intestine small, duodenum	+ + + + + + + + + + + + A A + + + + + + + + + +
Intestine small, jejunum	A + + + + + + + + + + + A A + + + + + + + A + +
Intestine small, ileum	+ + + + + + + + + A + + A A + + + + + + + + + +
Liver	+ +
Hepatocellular carcinoma	
Hepatocellular adenoma	
Histiocytic sarcoma	X X X X X X X
Mesentery	
Pancreas	+ +
Histiocytic sarcoma	X
Salivary glands	+ + + + + + + + + + + + + + + M + + + + + + + + +
Stomach, forestomach	+ +
Squamous cell papilloma	
Stomach, glandular	+ +
Cardiovascular System	
Heart	+ +
Alveolar/bronchiolar carcinoma, metastatic, lung	X
Endocrine System	
Adrenal cortex	+ +
Adrenal medulla	+ +
Islets, pancreatic	+ +
Parathyroid gland	+ + + + + M + + + + + + + M + + + + + + + + + + +
Pituitary gland	+ + + + + + + + + + + + + + + M + + + + + + + + +
Thyroid gland	+ +
General Body System	
None	
Genital System	
Clitoral gland	+ +
Ovary	+ +
Cystadenoma	X
Hemangioma	
Sex-cord stromal tumor, benign	X
Uterus	+ +
Polyp stromal	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm
(continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	3 7 1	3 7 2	3 7 3	3 7 5	3 7 6	3 7 7	3 7 9	3 8 1	3 8 2	3 8 4	3 8 5	3 9 3	3 9 5	3 9 6	4 0 2	4 0 3	4 0 4	4 0 9	4 1 2	4 1 5	3 7 4	3 9 1	4 0 0	4 0 5	4 0 8	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Histiocytic sarcoma																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma															X											1
Hepatocellular adenoma					X																					1
Histiocytic sarcoma																										7
Mesentery						+																				1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma	X			X										X												3
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma																										1
Hemangioma																									X	1
Sex-cord stromal tumor, benign													X					X		X		X				5
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal																X										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm
(continued)

Number of Days on Study	0 4 4 4 5 5 5 5 5 5 5 5 6 6 6 6 6 7 7 7 7 7 7 4 4 4 5 0 1 6 7 7 8 8 9 9 0 0 4 5 6 8 1 2 3 3 3 3 0 7 9 0 5 4 8 3 8 8 9 4 8 1 2 0 2 7 0 5 2 0 7 7 7
Carcass ID Number	4 3 3 3 4 4 3 3 3 3 3 3 3 3 4 3 3 4 3 4 3 4 3 3 3 1 9 8 9 1 1 9 9 8 8 9 9 8 7 0 8 6 1 8 0 6 0 6 6 7 4 8 0 9 1 0 4 7 8 3 2 0 9 8 1 7 9 3 6 6 8 7 6 7 0
Hematopoietic System	
Bone marrow	+ +
Histiocytic sarcoma	X X X X
Lymph node	+ + + + + + + + + + + + + + +
Iliac, histiocytic sarcoma	X X X
Mediastinal, histiocytic sarcoma	X
Pancreatic, histiocytic sarcoma	X
Renal, histiocytic sarcoma	X X
Lymph node, mandibular	+ + + + + + + + + + + + + + M + + + + + + + + +
Histiocytic sarcoma	X X X X
Lymph node, mesenteric	+ +
Histiocytic sarcoma	X X X X
Spleen	+ +
Hemangiosarcoma	X
Histiocytic sarcoma	X X X X X X
Thymus	+ M + + + + + + + + + + M + + + + + + + + + + + +
Alveolar/bronchiolar carcinoma, metastatic, lung	X
Histiocytic sarcoma	X X
Integumentary System	
Mammary gland	+ +
Skin	+ +
Subcutaneous tissue, hemangiosarcoma	X
Musculoskeletal System	
Bone	+ +
Osteosarcoma	X
Nervous System	
Brain	+ +
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	X
Alveolar/bronchiolar carcinoma	X
Histiocytic sarcoma	X X X X
Osteosarcoma, metastatic, bone	X
Nose	+ +
Carcinoma, metastatic, harderian gland	
Trachea	+ +
Special Senses System	
Ear	+ +
Harderian gland	+
Adenoma	X
Carcinoma	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm
(continued)

Number of Days on Study	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 7	7 3 8	7 3 8	7 3 8	7 3 8	
Carcass ID Number	3 7 1	3 7 2	3 7 3	3 7 5	3 7 6	3 7 7	3 7 9	3 8 1	3 8 2	3 8 4	3 8 5	3 9 3	3 9 5	3 9 6	4 0 2	4 0 3	4 0 4	4 0 9	4 1 2	4 1 5	3 7 4	3 9 1	4 0 0	4 0 5	4 0 8	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										5
Lymph node											+												+	+	+	16
Iliac, histiocytic sarcoma																										3
Mediastinal, histiocytic sarcoma																										1
Pancreatic, histiocytic sarcoma																										1
Renal, histiocytic sarcoma																										2
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										4
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										4
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Histiocytic sarcoma																										6
Thymus	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	45
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Histiocytic sarcoma																										2
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma									X								X	X				X	X			6
Alveolar/bronchiolar carcinoma												X														2
Histiocytic sarcoma																										4
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, harderian gland																		X								1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										2
Harderian gland																		+								2
Adenoma																										1
Carcinoma																		X								1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm
(continued)

Number of Days on Study	0 4 0	4 4 7	4 4 9	4 5 0	5 0 5	5 1 4	5 6 8	5 7 3	5 7 8	5 8 8	5 8 9	5 9 4	5 9 8	6 0 1	6 0 2	6 4 0	6 5 2	6 6 7	6 8 0	7 1 5	7 2 2	7 3 0	7 3 7	7 3 7
Carcass ID Number	4 1 4	3 9 8	3 8 0	3 9 9	4 1 1	4 1 0	3 9 4	3 9 7	3 8 8	3 8 3	3 9 2	3 9 0	3 8 9	3 7 8	4 0 1	3 8 7	3 6 9	4 1 3	3 8 6	4 0 6	3 6 8	4 0 7	3 6 6	3 7 7	3 7 0
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma												X												
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma										X		X	X	X	X		X					X		
Lymphoma malignant		X		X	X	X	X		X		X								X		X		X	X	X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of Phenolphthalein: 12,000 ppm
(continued)

Number of Days on Study	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	
	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	8	8	
Carcass ID Number	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	3	3	4	4	4	Total
	7	7	7	7	7	7	7	8	8	8	8	9	9	9	0	0	0	0	1	1	7	9	0	0	0	Tissues/
	1	2	3	5	6	7	9	1	2	4	5	3	5	6	2	3	4	9	2	5	4	1	0	5	8	Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										7
Lymphoma malignant			X	X	X	X			X		X			X			X		X		X			X	X	X	25

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

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Harderian Gland: Adenoma or Carcinoma				
Overall rate[a]	4/50 (8%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate[b]	10.0%	3.2%	2.9%	7.1%
Terminal rate[c]	3/39 (8%)	1/31 (3%)	1/34 (3%)	2/28 (7%)
First incidence (days)	730	737 (T)	737 (T)	737 (T)
Life table test[d]	P=0.422N	P=0.258N	P=0.227N	P=0.497N
Logistic regression test[d]	P=0.414N	P=0.259N	P=0.230N	P=0.485N
Cochran-Armitage test[d]	P=0.313N			
Fisher exact test[d]		P=0.181N	P=0.181N	P=0.339N
Liver: Hepatocellular Adenoma				
Overall rate	17/50 (34%)	2/50 (4%)	6/50 (12%)	1/50 (2%)
Adjusted rate	41.2%	6.5%	17.1%	3.6%
Terminal rate	15/39 (38%)	2/31 (6%)	5/34 (15%)	1/28 (4%)
First incidence (days)	597	737 (T)	693	737 (T)
Life table test	P<0.001N	P=0.001N	P=0.022N	P<0.001N
Logistic regression test	P<0.001N	P<0.001N	P=0.015N	P<0.001N
Cochran-Armitage test	P<0.001N			
Fisher exact test		P<0.001N	P=0.008N	P<0.001N
Liver: Hepatocellular Carcinoma				
Overall rate	6/50 (12%)	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adjusted rate	14.9%	3.2%	0.0%	3.6%
Terminal rate	5/39 (13%)	1/31 (3%)	0/34 (0%)	1/28 (4%)
First incidence (days)	708	737 (T)	—[e]	737 (T)
Life table test	P=0.046N	P=0.106N	P=0.028N	P=0.128N
Logistic regression test	P=0.042N	P=0.099N	P=0.025N	P=0.111N
Cochran-Armitage test	P=0.025N			
Fisher exact test		P=0.056N	P=0.013N	P=0.056N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	21/50 (42%)	3/50 (6%)	6/50 (12%)	2/50 (4%)
Adjusted rate	49.8%	9.7%	17.1%	7.1%
Terminal rate	18/39 (46%)	3/31 (10%)	5/34 (15%)	2/28 (7%)
First incidence (days)	597	737 (T)	693	737 (T)
Life table test	P<0.001N	P<0.001N	P=0.003N	P<0.001N
Logistic regression test	P<0.001N	P<0.001N	P=0.002N	P<0.001N
Cochran-Armitage test	P<0.001N			
Fisher exact test		P<0.001N	P<0.001N	P<0.001N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	5/50 (10%)	6/50 (12%)
Adjusted rate	7.2%	3.2%	14.7%	20.7%
Terminal rate	2/39 (5%)	1/31 (3%)	5/34 (15%)	5/28 (18%)
First incidence (days)	615	737 (T)	737 (T)	730
Life table test	P=0.032	P=0.385N	P=0.289	P=0.115
Logistic regression test	P=0.041	P=0.328N	P=0.315	P=0.154
Cochran-Armitage test	P=0.080			
Fisher exact test		P=0.309N	P=0.357	P=0.243

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	1/50 (2%)	2/50 (4%)
Adjusted rate	9.4%	12.3%	2.9%	5.6%
Terminal rate	2/39 (5%)	3/31 (10%)	0/34 (0%)	1/28 (4%)
First incidence (days)	597	663	693	449
Life table test	P=0.276N	P=0.514	P=0.231N	P=0.459N
Logistic regression test	P=0.190N	P=0.607	P=0.180N	P=0.321N
Cochran-Armitage test	P=0.186N			
Fisher exact test		P=0.643N	P=0.181N	P=0.339N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	6/50 (12%)	5/50 (10%)	6/50 (12%)	8/50 (16%)
Adjusted rate	13.8%	15.4%	17.1%	25.7%
Terminal rate	3/39 (8%)	4/31 (13%)	5/34 (15%)	6/28 (21%)
First incidence (days)	597	663	693	449
Life table test	P=0.133	P=0.597	P=0.522	P=0.198
Logistic regression test	P=0.213	P=0.542N	P=0.603	P=0.354
Cochran-Armitage test	P=0.269			
Fisher exact test		P=0.500N	P=0.620N	P=0.387
Ovary: Cystadenoma				
Overall rate	0/50 (0%)	3/49 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	9.7%	0.0%	2.1%
Terminal rate	0/39 (0%)	3/31 (10%)	0/34 (0%)	0/28 (0%)
First incidence (days)	—	737 (T)	—	449
Life table test	P=0.575	P=0.084	—	P=0.500
Logistic regression test	P=0.626N	P=0.084	—	P=0.518
Cochran-Armitage test	P=0.631N			
Fisher exact test		P=0.117	—	P=0.500
Ovary: Benign Sex-Cord Stromal Tumor				
Overall rate	0/50 (0%)	7/49 (14%)	6/50 (12%)	5/50 (10%)
Adjusted rate	0.0%	21.7%	17.6%	17.9%
Terminal rate	0/39 (0%)	6/31 (19%)	6/34 (18%)	5/28 (18%)
First incidence (days)	—	668	737 (T)	737 (T)
Life table test	P=0.056	P=0.004	P=0.011	P=0.012
Logistic regression test	P=0.066	P=0.004	P=0.011	P=0.012
Cochran-Armitage test	P=0.142			
Fisher exact test		P=0.006	P=0.013	P=0.028
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	3/49 (6%)	2/50 (4%)	1/49 (2%)	0/48 (0%)
Adjusted rate	7.9%	6.5%	2.9%	0.0%
Terminal rate	3/38 (8%)	2/31 (6%)	1/34 (3%)	0/27 (0%)
First incidence (days)	737 (T)	737 (T)	737 (T)	—
Life table test	P=0.094N	P=0.593N	P=0.345N	P=0.187N
Logistic regression test	P=0.094N	P=0.594N	P=0.345N	P=0.187N
Cochran-Armitage test	P=0.064N			
Fisher exact test		P=0.490N	P=0.309N	P=0.125N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	1/50 (2%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.6%	3.2%	0.0%	10.7%
Terminal rate	1/39 (3%)	1/31 (3%)	0/34 (0%)	3/28 (11%)
First incidence (days)	737 (T)	737 (T)	—	737 (T)
Life table test	P=0.105	P=0.710	P=0.527N	P=0.195
Logistic regression test	P=0.105	P=0.710	P=0.527N	P=0.195
Cochran-Armitage test	P=0.160			
Fisher exact test		P=0.753N	P=0.500N	P=0.309
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	1/50 (2%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.6%	6.5%	0.0%	10.7%
Terminal rate	1/39 (3%)	2/31 (6%)	0/34 (0%)	3/28 (11%)
First incidence (days)	737 (T)	737 (T)	—	737 (T)
Life table test	P=0.164	P=0.420	P=0.527N	P=0.195
Logistic regression test	P=0.164	P=0.420	P=0.527N	P=0.195
Cochran-Armitage test	P=0.242			
Fisher exact test		P=0.500	P=0.500N	P=0.309
Uterus: Stromal Polyp				
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.6%	6.2%	8.8%	3.6%
Terminal rate	1/39 (3%)	1/31 (3%)	3/34 (9%)	1/28 (4%)
First incidence (days)	737 (T)	703	737 (T)	737 (T)
Life table test	P=0.521	P=0.419	P=0.257	P=0.686
Logistic regression test	P=0.541	P=0.440	P=0.257	P=0.686
Cochran-Armitage test	P=0.577N			
Fisher exact test		P=0.500	P=0.309	P=0.753N
All Organs: Hemangiosarcoma				
Overall rate	0/50 (0%)	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	0.0%	3.2%	10.8%	3.2%
Terminal rate	0/39 (0%)	1/31 (3%)	3/34 (9%)	0/28 (0%)
First incidence (days)	—	737 (T)	567	715
Life table test	P=0.263	P=0.454	P=0.051	P=0.444
Logistic regression test	P=0.312	P=0.454	P=0.062	P=0.473
Cochran-Armitage test	P=0.337			
Fisher exact test		P=0.500	P=0.059	P=0.500
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	1/50 (2%)	4/50 (8%)	2/50 (4%)
Adjusted rate	4.8%	3.2%	10.8%	6.7%
Terminal rate	1/39 (3%)	1/31 (3%)	3/34 (9%)	1/28 (4%)
First incidence (days)	686	737 (T)	567	715
Life table test	P=0.368	P=0.583N	P=0.285	P=0.575
Logistic regression test	P=0.435	P=0.548N	P=0.332	P=0.640
Cochran-Armitage test	P=0.477			
Fisher exact test		P=0.500N	P=0.339	P=0.691N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
All Organs: Histiocytic Sarcoma				
Overall rate	0/50 (0%)	2/50 (4%)	7/50 (14%)	7/50 (14%)
Adjusted rate	0.0%	6.5%	17.6%	18.0%
Terminal rate	0/39 (0%)	2/31 (6%)	3/34 (9%)	0/28 (0%)
First incidence (days)	—	737 (T)	567	588
Life table test	P=0.002	P=0.189	P=0.007	P=0.005
Logistic regression test	P=0.009	P=0.189	P=0.010	P=0.011
Cochran-Armitage test	P=0.004			
Fisher exact test		P=0.247	P=0.006	P=0.006
All Organs: Malignant Lymphoma				
Overall rate	15/50 (30%)	28/50 (56%)	33/50 (66%)	25/50 (50%)
Adjusted rate	34.3%	60.2%	74.6%	65.8%
Terminal rate	11/39 (28%)	13/31 (42%)	23/34 (68%)	16/28 (57%)
First incidence (days)	156	266	338	447
Life table test	P=0.010	P=0.003	P<0.001	P=0.004
Logistic regression test	P=0.114	P=0.051	P<0.001	P=0.035
Cochran-Armitage test	P=0.060			
Fisher exact test		P=0.007	P<0.001	P=0.033
All Organs: Benign Neoplasms				
Overall rate	26/50 (52%)	20/50 (40%)	23/50 (46%)	17/50 (34%)
Adjusted rate	58.8%	58.7%	61.7%	56.1%
Terminal rate	21/39 (54%)	17/31 (55%)	20/34 (59%)	15/28 (54%)
First incidence (days)	78	663	479	449
Life table test	P=0.372N	P=0.509N	P=0.566	P=0.389N
Logistic regression test	P=0.208N	P=0.431N	P=0.559N	P=0.199N
Cochran-Armitage test	P=0.067N			
Fisher exact test		P=0.158N	P=0.345N	P=0.053N
All Organs: Malignant Neoplasms				
Overall rate	25/50 (50%)	35/50 (70%)	41/50 (82%)	37/50 (74%)
Adjusted rate	55.1%	71.4%	85.4%	78.5%
Terminal rate	19/39 (49%)	17/31 (55%)	27/34 (79%)	18/28 (64%)
First incidence (days)	156	266	338	447
Life table test	P=0.002	P=0.011	P<0.001	P=0.001
Logistic regression test	P=0.040	P=0.166	P<0.001	P=0.058
Cochran-Armitage test	P=0.010			
Fisher exact test		P=0.033	P<0.001	P=0.011

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	40/50 (80%)	47/50 (94%)	43/50 (86%)
Adjusted rate	83.3%	81.6%	95.9%	91.4%
Terminal rate	31/39 (79%)	22/31 (71%)	32/34 (94%)	24/28 (86%)
First incidence (days)	78	266	338	447
Life table test	P=0.014	P=0.135	P=0.024	P=0.014
Logistic regression test	P=0.230	P=0.566N	P=0.037	P=0.282
Cochran-Armitage test	P=0.158			
Fisher exact test		P=0.598N	P=0.036	P=0.298

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, pituitary gland, stomach, and uterus; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, 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 Histiocytic Sarcoma in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls
Historical Incidence at EG&G Mason Research Institute/TSI Mason Laboratories, Inc.	
1-Amino-2,4-dibromoanthraquinone	0/50
Acetaminophen	1/50
HC Yellow 4	0/50
Methylphenidate Hydrochloride	2/49
Pentaerythritol Tetranitrate	0/50
Turmeric Oleoresin	0/50
Overall Historical Incidence	
Total	19/1,473 (1.3%)
Standard deviation	1.6%
Range	0%-4%

[a] Data as of 12 May 1995

TABLE D4b
Historical Incidence of Malignant Lymphoma in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls
Historical Incidence at EG&G Mason Research Institute/TSI Mason Laboratories, Inc.	
1-Amino-2,4-dibromoanthraquinone	11/50
Acetaminophen	10/50
HC Yellow 4	9/50
Methylphenidate Hydrochloride	12/49
Pentaerythritol Tetranitrate	20/50
Turmeric Oleoresin	9/50
Overall Historical Incidence	
Total	339/1,473 (23.0%)
Standard deviation	11.9%
Range	6%-44%

[a] Data as of 12 May 1995; includes data for histocytic, lymphocytic, mixed, unspecified, or undifferentiated cell type lymphomas

TABLE D4c
Historical Incidence of Luteoma in the Ovary of Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls
Historical Incidence at EG&G Mason Research Institute/TSI Mason Laboratories, Inc.	
1-Amino-2,4-dibromoanthraquinone	0/49
Acetaminophen	0/47
HC Yellow 4	0/50
Methylphenidate Hydrochloride	0/46
Pentaerythritol Tetranitrate	0/50
Turmeric Oleoresin	0/50
Overall Historical Incidence	
Total	5/1,436 (0.4%)
Standard deviation	1.0%
Range	0%-4%

[a] Data as of 12 May 1995

TABLE D4d
Historical Incidence of Hepatocellular Neoplasms in Untreated Female B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at EG&G Mason Research Institute/TSI Mason Laboratories, Inc.			
1-Amino-2,4-dibromoanthraquinone	6/50	0/50	6/50
Acetaminophen	3/49	0/49	3/49
HC Yellow 4	5/50	1/50	6/50
Methylphenidate Hydrochloride	6/49	5/49	9/49
Pentaerythritol Tetranitrate	5/49	1/49	6/49
Turmeric Oleoresin	7/50	7/50	13/50
Overall Historical Incidence			
Total	231/1,464 (15.8%)	108/1,464 (7.4%)	313/1,464 (21.4%)
Standard deviation	10.6%	6.1%	13.0%
Range	2%-50%	0%-20%	3%-56%

[a] Data as of 12 May 1995

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

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	13	9	12
Natural deaths	6	6	7	10
Survivors				
Died last week of the study	1			
Terminal sacrifice	38	31	34	28
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(50)	(48)	(48)	(45)
Hemorrhage			1 (2%)	
Epithelium, hyperplasia				1 (2%)
Intestine small, jejunum	(49)	(48)	(46)	(46)
Hemorrhage			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)			4 (8%)
Clear cell focus	1 (2%)	2 (4%)		
Eosinophilic focus	20 (40%)	4 (8%)	2 (4%)	1 (2%)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation			2 (4%)	1 (2%)
Inflammation, chronic	1 (2%)		1 (2%)	
Mixed cell focus	3 (6%)	2 (4%)	1 (2%)	
Necrosis	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Vacuolization cytoplasmic	3 (6%)			2 (4%)
Centrilobular, necrosis				1 (2%)
Kupffer cell, hyperplasia	1 (2%)			
Mesentery	(16)	(2)	(7)	(1)
Inflammation, chronic	1 (6%)			
Fat, congestion			1 (14%)	
Fat, necrosis	14 (88%)	2 (100%)	5 (71%)	1 (100%)
Pancreas	(50)	(50)	(50)	(50)
Cytoplasmic alteration				1 (2%)
Inflammation, acute				1 (2%)
Inflammation, chronic	1 (2%)			
Necrosis				1 (2%)
Acinus, atrophy				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema			2 (4%)	
Hemorrhage				1 (2%)
Hyperkeratosis		1 (2%)		
Hyperplasia, squamous	3 (6%)	6 (12%)	4 (8%)	2 (4%)
Inflammation, chronic		1 (2%)	1 (2%)	
Ulcer		2 (4%)		3 (6%)
Muscularis, hyperplasia	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 Phenolphthalein
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Alimentary System (continued)				
Stomach, glandular	(50)	(49)	(49)	(50)
Edema			2 (4%)	
Erosion		1 (2%)	1 (2%)	3 (6%)
Glands, hyperplasia			1 (2%)	
Tooth			(1)	
Inflammation, chronic			1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		1 (2%)
Cardiomyopathy	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Hemorrhage			1 (2%)	1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)		
Inflammation, chronic active				1 (2%)
Mineralization		5 (10%)	4 (8%)	7 (14%)
Atrium, thrombosis				1 (2%)
Valve, inflammation, acute			1 (2%)	
Valve, inflammation, chronic active		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Degeneration, cystic				1 (2%)
Hematopoietic cell proliferation	1 (2%)			1 (2%)
Hypertrophy, focal	1 (2%)			
Capsule, hyperplasia	49 (98%)	50 (100%)	50 (100%)	49 (98%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)			
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia	32 (65%)	32 (64%)	37 (74%)	26 (52%)
Parathyroid gland	(46)	(48)	(47)	(47)
Cyst	1 (2%)			
Hyperplasia			1 (2%)	
Pituitary gland	(49)	(50)	(49)	(48)
Pars distalis, angiectasis		1 (2%)		
Pars distalis, cyst		1 (2%)		1 (2%)
Pars distalis, hyperplasia	11 (22%)	4 (8%)	1 (2%)	1 (2%)
Rathke's cleft, cyst	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Inflammation, chronic	1 (2%)			
Follicular cell, hyperplasia	27 (54%)	8 (16%)	3 (6%)	7 (14%)
General Body System				
None				

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Phenolphthalein
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Genital System				
Clitoral gland	(48)	(48)	(48)	(50)
Cyst	2 (4%)	2 (4%)		1 (2%)
Inflammation, chronic active			1 (2%)	
Pigmentation	2 (4%)	3 (6%)		1 (2%)
Ovary	(50)	(49)	(50)	(50)
Angiectasis	1 (2%)		1 (2%)	
Cyst	15 (30%)	8 (16%)	16 (32%)	12 (24%)
Hemorrhage	3 (6%)	1 (2%)	3 (6%)	2 (4%)
Hyperplasia	3 (6%)	10 (20%)	10 (20%)	17 (34%)
Hyperplasia, papillary	1 (2%)	1 (2%)		
Inflammation, chronic	1 (2%)			
Necrosis		1 (2%)		
Thrombosis				1 (2%)
Periovarian tissue, necrosis				1 (2%)
Uterus	(50)	(50)	(50)	(50)
Angiectasis	3 (6%)			
Hemorrhage			1 (2%)	
Hyperplasia, cystic	49 (98%)	46 (92%)	43 (86%)	45 (90%)
Inflammation, chronic active	1 (2%)			
Necrosis				1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia, reticulum cell		1 (2%)	1 (2%)	
Myelofibrosis	34 (68%)	34 (68%)	38 (76%)	36 (72%)
Pigmentation	2 (4%)	3 (6%)	11 (22%)	11 (22%)
Lymph node	(14)	(17)	(13)	(16)
Iliac, hyperplasia, lymphoid	2 (14%)			
Mediastinal, hyperplasia, lymphoid	1 (7%)			
Mediastinal, infiltration cellular, histiocyte	1 (7%)			
Mediastinal, pigmentation	1 (7%)			
Renal, infiltration cellular, histiocyte	1 (7%)			
Renal, pigmentation	1 (7%)			
Lymph node, mandibular	(49)	(50)	(49)	(49)
Atrophy				1 (2%)
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia, lymphoid		1 (2%)	1 (2%)	
Lymph node, mesenteric	(49)	(50)	(49)	(50)
Angiectasis	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Atrophy				1 (2%)
Hematopoietic cell proliferation	1 (2%)			
Hemorrhage	1 (2%)			
Hyperplasia, lymphoid	1 (2%)		1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation	13 (26%)	14 (28%)	20 (40%)	21 (42%)
Pigmentation	4 (8%)	1 (2%)	2 (4%)	3 (6%)
Lymphoid follicle, atrophy		1 (2%)		1 (2%)
Lymphoid follicle, hyperplasia	6 (12%)	5 (10%)	3 (6%)	

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Phenolphthalein
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Hematopoietic System (continued)				
Thymus	(48)	(44)	(49)	(45)
Atrophy	3 (6%)	1 (2%)	6 (12%)	9 (20%)
Fibrosis	1 (2%)			
Hyperplasia, atypical		7 (16%)	6 (12%)	5 (11%)
Infiltration cellular, polymorphonuclear	2 (4%)			
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Inflammation, chronic		2 (4%)		
Ulcer				1 (2%)
Subcutaneous tissue, edema			3 (6%)	3 (6%)
Subcutaneous tissue, inflammation, chronic				1 (2%)
Musculoskeletal System				
None				
Nervous System				
Brain	(50)	(49)	(50)	(50)
Demyelination				1 (2%)
Hemorrhage				2 (4%)
Thalamus, mineralization	28 (56%)	23 (47%)	24 (48%)	24 (48%)
Peripheral nerve	(1)		(1)	
Demyelination	1 (100%)			
Spinal cord	(1)		(1)	
Demyelination	1 (100%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	7 (14%)	4 (8%)	6 (12%)	4 (8%)
Infiltration cellular, histiocyte	2 (4%)	3 (6%)		
Thrombosis	1 (2%)			
Alveolar epithelium, hyperplasia	1 (2%)		1 (2%)	1 (2%)
Special Senses System				
None				

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Phenolphthalein
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst		1 (2%)		1 (2%)
Hydronephrosis	1 (2%)	1 (2%)		1 (2%)
Infarct		1 (2%)		
Inflammation, chronic	1 (2%)			
Mineralization			1 (2%)	
Necrosis			1 (2%)	1 (2%)
Nephropathy, chronic	15 (30%)	16 (32%)	19 (38%)	14 (28%)
Pigmentation	1 (2%)			
Pelvis, hemorrhage				1 (2%)
Renal tubule, accumulation, hyaline droplet		1 (2%)	3 (6%)	8 (16%)
Renal tubule, dilatation				2 (4%)
Urinary bladder	(50)	(50)	(49)	(50)
Hyperplasia, lymphoid		1 (2%)		
Infiltration cellular, lymphocyte			1 (2%)	

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Mortelmans *et al.* (1986). Phenolphthalein was sent to the laboratories as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of phenolphthalein. The high dose was limited by toxicity. All trials were repeated.

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

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987) and by Witt *et al.* (1995). Phenolphthalein was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs) both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of phenolphthalein; the high dose was limited by toxicity. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with phenolphthalein in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing phenolphthalein was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with phenolphthalein, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no phenolphthalein, and incubation proceeded for an additional 26 hours with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind, and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level. Because significant chemical-induced cell cycle delay was seen at the 50 μg/mL dose, incubation time was lengthened to 31 hours to ensure a sufficient number of scorable (second-division metaphase) cells.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A highly significant trend ($P \leq 0.005$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with phenolphthalein for 14.2 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with phenolphthalein and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 12 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test; because cell cycle delay was anticipated in the absence of S9, the incubation period for these cultures was extended.

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

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend in the absence of a statistically significant increase at any one dose point led to an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented in Dietz *et al.* (1992). At the end of the 13-week study, blood was obtained from male and female mice, and smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with a chromatin-specific fluorescent dye mixture of Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983) and coded. Slides were scanned at a magnification of 630× or 1000× using a semi-automated image analysis system to determine the frequency of micronuclei in 2,000 polychromatic erythrocytes (PCEs) and 10,000 normochromatic erythrocytes (NCEs) in each of 10 animals per exposure group. The criteria of Schmid (1976) were used to define micronuclei, with the additional requirement that micronuclei exhibit the characteristic fluorescent emissions of DNA (blue with 360 nm and orange with 540 nm illumination); the minimum size limit was approximately one-twentieth the diameter of the nucleus.

The frequency of micronucleated cells among PCEs was analyzed by the Cochran-Armitage trend test, and individual exposure groups were compared to the concurrent control groups by Kastenbaum-Bowman's binomial test. Log transformation of the NCE data and testing for normality by the Shapiro-Wilk test and for heterogeneity of variance by Cochran's test were performed before statistical analyses. The frequency

of micronucleated cells among NCEs was analyzed by analysis of variance using the SAS GLM procedure. The NCE data for each exposure group were compared with the concurrent control group using Student's *t*-test.

RESULTS

Phenolphthalein, tested independently in two laboratories, was not mutagenic in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without induced rat or hamster liver S9 metabolic activation enzymes (Table E1; Mortelmans *et al.*, 1986). In cytogenetic tests with cultured CHO cells, no induction of SCEs was observed after treatment with 0.5 to 50 μg/mL phenolphthalein with or without S9 (Table E2). Cytotoxicity was noted at the 50 μg/mL dose level, and culture times were extended to maximize the proportion of cells available for analysis at this dose level. Results of the *in vitro* Abs test with phenolphthalein were positive for the two trials conducted with S9; no increase in Abs was observed in the absence of S9 (Table E3; Witt *et al.*, 1995). At the 50 μg/mL dose level, approximately one quarter of all cells contained aberrations, and most of these were chromosome breaks located at the distal end of X_q. The significance of this preferential breakage is as yet unknown, but it has been noted with other chemicals, and it occurs only in the presence of S9. A discussion of possible mechanisms for this phenomenon is presented in Galloway *et al.* (1987). Results from the *in vivo* mouse peripheral blood micronucleus test were positive for both male and female mice (Table E4; Dietz *et al.*, 1992). Significant increases in micronuclei were noted for both NCE and PCE populations. The increases in micronucleated NCEs, which indicate the effects of chronic exposure, occurred at all exposure concentrations in males and females. The effects on PCEs, which indicate chromosomal damage induced within the 48 hours preceding analysis, occurred only at 25,000 and 50,000 ppm, the two highest exposure concentrations.

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

Strain	Dose (μg/plate)	Revertants/plate[b] −S9 Trial 1	−S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at EG&G Mason Research Institute							
TA100	0	153 ± 7.9	176 ± 6.2	144 ± 5.7	197 ± 6.9	125 ± 5.6	169 ± 6.8
	3.3	141 ± 7.2	170 ± 4.5	140 ± 6.6	196 ± 7.5	124 ± 3.0	173 ± 11.8
	10	131 ± 2.1	180 ± 4.7	145 ± 8.3	211 ± 8.5	150 ± 7.6	166 ± 14.7
	33	127 ± 3.0	163 ± 3.4	140 ± 10.4	198 ± 7.2	140 ± 9.9	177 ± 1.5
	100	157 ± 7.4	184 ± 9.0	162 ± 1.5	204 ± 8.8	148 ± 6.6	191 ± 7.2
	220		132 ± 2.7[c]		226 ± 3.5[c]		168 ± 6.9[c]
	333	Toxic		94 ± 6.4[c]		92 ± 5.9[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		984 ± 41.6	1,072 ± 48.0	1,109 ± 32.7	691 ± 21.4	908 ± 46.0	617 ± 8.8
TA1535	0	31 ± 3.8	28 ± 7.0	13 ± 1.2	11 ± 1.2	15 ± 1.9	14 ± 0.6
	3.3	29 ± 3.5	26 ± 1.9	11 ± 0.0	12 ± 0.0	10 ± 1.8	12 ± 2.3
	10	21 ± 4.7	19 ± 3.3	9 ± 2.0	12 ± 1.9	11 ± 3.3	11 ± 0.7
	33	31 ± 5.2	21 ± 3.7	9 ± 1.0	13 ± 4.2	9 ± 3.8	17 ± 1.8
	100	28 ± 4.0	25 ± 2.6	11 ± 0.9	14 ± 0.6	12 ± 2.3	13 ± 1.2
	220		Toxic		14 ± 0.6[c]		15 ± 4.7[c]
	333	Toxic		Toxic		6 ± 1.0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		972 ± 21.3	840 ± 53.4	148 ± 15.2	68 ± 1.5	117 ± 6.6	59 ± 0.9
TA1537	0	5 ± 2.3	7 ± 1.2	12 ± 1.8	9 ± 2.0	7 ± 1.5	9 ± 2.1
	3.3	7 ± 0.6	6 ± 2.3	12 ± 0.3	9 ± 2.2	7 ± 1.0	8 ± 0.3
	10	7 ± 2.3	7 ± 0.7	8 ± 1.2	7 ± 1.2	9 ± 0.3	10 ± 1.0
	33	6 ± 0.6	5 ± 2.0	8 ± 1.8	11 ± 1.2	8 ± 1.2	7 ± 0.6
	100	6 ± 1.2	11 ± 1.5	9 ± 1.5	9 ± 0.3	8 ± 0.9	11 ± 1.8
	220		4 ± 1.0[c]		10 ± 1.8[c]		5 ± 0.3[c]
	333	Toxic		5 ± 1.7[c]		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		393 ± 85.6	188 ± 17.2	102 ± 2.3	54 ± 2.5	73 ± 1.5	42 ± 2.5
TA98	0	17 ± 1.8	19 ± 2.7	23 ± 2.7	23 ± 4.0	19 ± 1.3	22 ± 2.3
	3.3	21 ± 2.1	18 ± 2.3	29 ± 3.2	23 ± 1.0	26 ± 0.9	28 ± 3.1
	10	20 ± 2.1	12 ± 2.0	26 ± 1.5	32 ± 2.1	23 ± 2.6	25 ± 2.6
	33	21 ± 2.7	14 ± 3.8	27 ± 2.0	25 ± 2.0	25 ± 4.5	25 ± 0.6
	100	16 ± 1.5	11 ± 0.9	29 ± 2.4	25 ± 1.2	25 ± 4.4	20 ± 0.3
	220		10 ± 2.9[c]		33 ± 5.0[c]		25 ± 2.6[c]
	333	Toxic		30 ± 2.4[c]		29 ± 2.6[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,335 ± 20.7	1,335 ± 27.2	1,231 ± 9.5	467 ± 30.1	1,001 ± 10.1	402 ± 49.7

TABLE E1
Mutagenicity of Phenolphthalein in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate −S9 Trial 1	−S9 Trial 2	−S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3
Study performed at Case Western Reserve University							
TA100	0	86 ± 1.7	99 ± 10.2	87 ± 0.9	90 ± 6.5	104 ± 8.0	113 ± 12.8
	3.3		94 ± 6.0	82 ± 1.2		94 ± 2.4	96 ± 5.6
	10	94 ± 8.0	79 ± 7.7	88 ± 4.3	95 ± 8.0	95 ± 3.8	102 ± 10.1
	33	90 ± 6.0	85 ± 5.9	82 ± 7.8	102 ± 6.0	113 ± 9.0	89 ± 6.7
	100	109 ± 23.4	72 ± 1.2	70 ± 7.3	100 ± 9.2	120 ± 5.8	108 ± 10.7
	333	Toxic	Toxic	Toxic	Toxic	122 ± 6.9	117 ± 9.3
	1,000	Toxic			13 ± 8.5		
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,381 ± 22.1	911 ± 36.0	1,641 ± 38.6	945 ± 126.4	1,383 ± 108.6	2,007 ± 217.0

Strain	Dose (μg/plate)	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100 (continued)	0	115 ± 9.9	128 ± 3.5
	3.3		
	10	92 ± 5.9	107 ± 2.7
	33	90 ± 1.8	128 ± 2.9
	100	101 ± 3.5	118 ± 6.2
	333	90 ± 3.9	126 ± 4.0
	1,000	Toxic	103 ± 15.4
Trial summary		Negative	Negative
Positive control		1,491 ± 345.7	1,586 ± 45.0

Strain	Dose (μg/plate)	−S9 Trial 1	−S9 Trial 2	−S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2
TA1535	0	7 ± 0.9	14 ± 1.5	5 ± 1.8	10 ± 1.0	13 ± 0.3
	3.3		12 ± 1.2	6 ± 1.7		
	10	10 ± 0.9	9 ± 1.5	7 ± 1.5	13 ± 1.5	12 ± 3.5
	33	9 ± 2.3	8 ± 1.5	5 ± 0.7	9 ± 1.2	10 ± 1.7
	100	9 ± 1.0	10 ± 0.7	4 ± 1.5	13 ± 2.3	9 ± 2.4
	333	Toxic	9 ± 1.8	Toxic	11 ± 3.7	11 ± 2.0
	1,000	Toxic			Toxic	12 ± 3.3
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		1,029 ± 16.2	680 ± 28.7	1,081 ± 144.9	308 ± 26.5	133 ± 6.8

TABLE E1
Mutagenicity of Phenolphthalein in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate +10% rat S9 Trial 1	Revertants/plate +10% rat S9 Trial 2
Study performed at Case Western Reserve University (continued)			
TA1535 (continued)	0	10 ± 4.0	11 ± 1.2
	3.3		
	10	6 ± 2.6	11 ± 1.9
	33	7 ± 1.2	7 ± 1.0
	100	9 ± 0.0	11 ± 2.1
	333	8 ± 1.7	10 ± 2.1
	1,000	Toxic	16 ± 2.3
Trial summary		Negative	Negative
Positive control		416 ± 46.5	287 ± 19.9

		−S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA1537	0	3 ± 1.2	9 ± 2.6	13 ± 1.0	11 ± 1.5	10 ± 1.0	15 ± 1.0
	10	4 ± 0.3	9 ± 1.8	10 ± 3.2	9 ± 1.5	9 ± 2.2	11 ± 0.6
	33	7 ± 1.8	9 ± 0.9	9 ± 2.0	12 ± 0.9	9 ± 2.0	11 ± 1.2
	100	9 ± 1.5	10 ± 1.2	11 ± 0.3	9 ± 0.3	7 ± 1.7	13 ± 1.8
	333	4 ± 2.4	7 ± 0.9	10 ± 1.2	9 ± 2.1	8 ± 1.3	13 ± 1.8
	1,000	Toxic	Toxic	Toxic	Toxic	10 ± 3.5	Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		109 ± 9.6	113 ± 39.5	68 ± 9.6	194 ± 5.9	105 ± 8.0	158 ± 41.7
TA98	0	16 ± 1.8	25 ± 2.2	25 ± 3.4	30 ± 4.3	24 ± 1.2	31 ± 5.8
	10	13 ± 3.8	21 ± 2.9	22 ± 4.8	30 ± 4.2	22 ± 6.0	30 ± 1.5
	33	11 ± 1.5	21 ± 3.5	20 ± 3.5	27 ± 2.0	18 ± 2.7	31 ± 2.3
	100	17 ± 0.9	20 ± 4.1	29 ± 2.3	31 ± 0.9	19 ± 3.7	22 ± 3.5
	333	16 ± 3.8	17 ± 0.9	25 ± 2.6	23 ± 4.0	25 ± 3.5	19 ± 2.8
	1,000	Toxic	Toxic	Toxic	Toxic	Toxic	Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		146 ± 17.8	92 ± 4.0	554 ± 93.2	966 ± 33.4	334 ± 132.8	681 ± 66.8

[a] The detailed protocol and these data are presented in Mortelmans *et al.* (1986).
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Phenolphthalein[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
−S9								
Summary: Negative								
Dimethylsulfoxide		50	1,043	392	0.37	7.8	26.0	
Mitomycin-C	0.001	50	1,045	677	0.64	13.5	26.0	72.38
	0.005	10	212	277	1.30	27.7	26.0	247.66
Phenolphthalein	0.5	50	1,047	416	0.39	8.3	26.0	5.72
	1.7	50	1,050	427	0.40	8.5	26.0	8.20
	5	50	1,044	379	0.36	7.6	26.0	−3.41
	17	50	1,047	417	0.39	8.3	26.0	5.97
	50	0[c]					31.0[d]	
					P=0.419[e]			
+S9								
Summary: Negative								
Dimethylsulfoxide		50	1,042	407	0.39	8.1	26.0	
Cyclophosphamide	0.125	50	1,043	556	0.53	11.1	26.0	36.48
	0.5	10	208	170	0.81	17.0	26.0	109.25
Phenolphthalein	1.7	50	1,047	389	0.37	7.8	26.0	−4.88
	5	50	1,050	383	0.36	7.7	26.0	−6.61
	17	50	1,042	361	0.34	7.2	26.0	−11.30
	50	50	1,042	464	0.44	9.3	31.0[d]	14.00
					P=0.085			

[a] Study performed at SITEK Research Laboratories. A detailed description of the protocol is presented in Galloway *et al.* (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine.
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Dose was cytostatic.
[d] Because phenolphthalein induced a delay in the cell division cycle, harvest time was extended to maximize the proportion of second-division metaphase cells available for analysis.
[e] Significance of relative SCEs/chromosome tested by the linear regression trend test versus log of the dose

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Phenolphthalein[a]

−S9					+S9				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Harvest time: 16.2 hours[b]					**Trial 1** - Harvest time: 14.0 hours				
Summary: Negative					Summary: Weakly positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	200	2	0.01	1.0		200	3	0.02	1.0
Mitomycin-C					Cyclophosphamide				
0.1	200	67	0.34	25.0	5	200	47	0.24	17.0
0.4	50	37	0.74	46.0	15	50	32	0.64	34.0
Phenolphthalein					Phenolphthalein				
11	200	0	0.00	0	11	200	1	0.01	0.5
23	200	2	0.01	1.0	23	200	0	0	0
50	200	3	0.02	1.5	50	200	59	0.30	25.0*
				P=0.198[c]					P≤0.001
					Trial 2 - Harvest time: 14.0 hours				
					Summary: Positive				
					Dimethylsulfoxide				
						200	2	0.01	1.0
					Cyclophosphamide				
					5	200	27	0.14	13.0
					15	50	36	0.72	26.0
					Phenolphthalein				
					30	200	3	0.02	1.5
					40	200	9	0.05	4.5*
					50	200	77	0.39	29.0*
									P≤0.001

* Positive (P≤0.05)

[a] Study performed at SITEK Research Laboratories. The detailed protocol and these data are presented in Witt *et al.* (1995). Abs=aberrations.

[b] Because of chemical-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened to provide sufficient second-division metaphase cells at harvest.

[c] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose

TABLE E4
Frequency of Micronuclei in Mouse Peripheral Blood Erythrocytes Following Treatment with Phenolphthalein in Feed for 13 Weeks[a]

	Dose (ppm)	Micronucleated Cells/1,000 Cells[b] PCEs	NCEs	Number of Mice
Male	0	2.01 ± 0.26	1.75 ± 0.16	10
	6,000	2.08 ± 0.49	2.51 ± 0.23**	10
	12,000	2.29 ± 0.52	2.63 ± 0.24**	10
	25,000	5.36 ± 0.67**	4.03 ± 0.48**	10
	50,000	4.99 ± 0.56**	4.72 ± 0.25**	10
		P≤0.001[c]	P≤0.001	
Female	0	1.49 ± 0.24	1.32 ± 0.08	10
	6,000	2.20 ± 0.44	2.07 ± 0.19**	10
	12,000	2.93 ± 0.37	2.94 ± 0.23**	10
	25,000	5.36 ± 0.70**	4.41 ± 0.23**	10
	50,000	4.94 ± 0.64**	4.31 ± 0.35**	10
		P≤0.001	P≤0.001	

** P≤0.01 by Kastenbaum-Bowman's binomial test (PCEs) or Student's *t*-test (NCEs)
[a] Study performed at USDA Western Regional Center. The detailed protocol and these data are presented in Dietz *et al.* (1992); PCEs=polychromatic erythrocytes; NCEs=normochromatic erythrocytes; 2,000 PCEs and 10,000 NCEs scored per animal.
[b] Data are presented as mean ± standard error.
[c] Significance by Cochran-Armitage linear regression of proportions (PCEs) or linear contrasts from analysis of variance (NCEs)

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

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Study of Phenolphthalein[a]

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	360 ± 6	356 ± 7	360 ± 6	356 ± 6	359 ± 10	352 ± 6
Brain						
Absolute	2.033 ± 0.035	1.995 ± 0.025	1.995 ± 0.017	2.005 ± 0.026	2.005 ± 0.018	2.015 ± 0.033
Relative	5.65 ± 0.11	5.61 ± 0.09	5.56 ± 0.08	5.64 ± 0.08	5.62 ± 0.13	5.74 ± 0.09
Heart						
Absolute	1.115 ± 0.016	1.115 ± 0.029	1.127 ± 0.016	1.147 ± 0.033	1.141 ± 0.036	1.099 ± 0.021
Relative	3.10 ± 0.05	3.13 ± 0.06	3.14 ± 0.06	3.22 ± 0.09	3.19 ± 0.07	3.13 ± 0.04
R. Kidney						
Absolute	1.344 ± 0.032	1.309 ± 0.034	1.351 ± 0.023	1.439 ± 0.036	1.416 ± 0.039	1.464 ± 0.044
Relative	3.73 ± 0.05	3.67 ± 0.06	3.76 ± 0.06	4.04 ± 0.07**	3.95 ± 0.06	4.16 ± 0.08**
Liver						
Absolute	13.664 ± 0.535	13.245 ± 0.337	14.108 ± 0.491	15.266 ± 0.480	15.560 ± 0.507*	16.577 ± 0.485**
Relative	37.82 ± 0.83	37.18 ± 0.49	39.17 ± 1.00	42.85 ± 1.10**	43.39 ± 0.74**	47.16 ± 1.06**
Lung						
Absolute	1.916 ± 0.085	1.940 ± 0.114	1.933 ± 0.138	1.847 ± 0.087	1.745 ± 0.063	1.741 ± 0.109
Relative	5.34 ± 0.28	5.46 ± 0.33	5.40 ± 0.43	5.19 ± 0.24	4.88 ± 0.17	4.97 ± 0.33
R. Testis						
Absolute	1.481 ± 0.029	1.511 ± 0.031	1.488 ± 0.025	1.502 ± 0.030	1.525 ± 0.042	1.517 ± 0.029
Relative	4.11 ± 0.05	4.25 ± 0.05	4.14 ± 0.06	4.22 ± 0.06	4.26 ± 0.10	4.32 ± 0.08
Thymus						
Absolute	0.330 ± 0.014	0.414 ± 0.019**	0.346 ± 0.010	0.349 ± 0.018	0.324 ± 0.016	0.335 ± 0.017
Relative	0.92 ± 0.04	1.16 ± 0.04**	0.96 ± 0.03	0.98 ± 0.04	0.90 ± 0.03	0.95 ± 0.05

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Study of Phenolphthalein
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Female						
n	10	10	10	10	10	10
Necropsy body wt	210 ± 3	207 ± 4	212 ± 3	205 ± 3	202 ± 3	198 ± 3*
Brain						
Absolute	1.875 ± 0.017	1.885 ± 0.015	1.881 ± 0.019	1.861 ± 0.013	1.843 ± 0.016	1.816 ± 0.043
Relative	8.96 ± 0.08	9.12 ± 0.19	8.88 ± 0.08	9.10 ± 0.10	9.13 ± 0.10	9.16 ± 0.15
Heart						
Absolute	0.720 ± 0.015	0.728 ± 0.021	0.728 ± 0.021	0.722 ± 0.017	0.722 ± 0.014	0.710 ± 0.016
Relative	3.44 ± 0.05	3.51 ± 0.08	3.44 ± 0.09	3.53 ± 0.08	3.57 ± 0.07	3.58 ± 0.05
R. Kidney						
Absolute	0.806 ± 0.016	0.798 ± 0.014	0.817 ± 0.027	0.809 ± 0.013	0.807 ± 0.022	0.800 ± 0.022
Relative	3.85 ± 0.07	3.86 ± 0.08	3.85 ± 0.10	3.95 ± 0.05	3.99 ± 0.08	4.04 ± 0.10
Liver						
Absolute	6.911 ± 0.122	7.055 ± 0.214	7.110 ± 0.196	7.315 ± 0.336	7.423 ± 0.214	7.244 ± 0.179
Relative	32.98 ± 0.33	34.07 ± 0.93	33.52 ± 0.66	35.66 ± 1.35	36.68 ± 0.73*	36.54 ± 0.62*
Lung						
Absolute	1.385 ± 0.050	1.224 ± 0.030	1.445 ± 0.103	1.273 ± 0.039	1.199 ± 0.045	1.247 ± 0.070
Relative	6.64 ± 0.30	5.92 ± 0.17	6.80 ± 0.43	6.22 ± 0.20	5.93 ± 0.22	6.29 ± 0.32
Thymus						
Absolute	0.346 ± 0.013	0.322 ± 0.014	0.339 ± 0.015	0.299 ± 0.016	0.334 ± 0.016	0.292 ± 0.013*
Relative	1.66 ± 0.07	1.56 ± 0.08	1.60 ± 0.06	1.46 ± 0.08	1.65 ± 0.06	1.47 ± 0.06

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

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Study of Phenolphthalein[a]

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	28.8 ± 0.8	27.4 ± 0.5	28.4 ± 0.6	28.5 ± 0.6	29.3 ± 0.8	28.0 ± 0.6
Brain						
Absolute	0.474 ± 0.006	0.479 ± 0.013	0.481 ± 0.006	0.479 ± 0.005	0.494 ± 0.022	0.468 ± 0.005
Relative	16.52 ± 0.32	17.52 ± 0.42	17.03 ± 0.39	16.85 ± 0.32	16.91 ± 0.74	16.74 ± 0.31
Heart						
Absolute	0.155 ± 0.005	0.149 ± 0.007	0.145 ± 0.002	0.151 ± 0.004	0.149 ± 0.004	0.144 ± 0.004
Relative	5.39 ± 0.09	5.46 ± 0.26	5.13 ± 0.10	5.30 ± 0.12	5.11 ± 0.11	5.14 ± 0.11
R. Kidney						
Absolute	0.307 ± 0.011	0.292 ± 0.009	0.337 ± 0.026	0.301 ± 0.009	0.331 ± 0.014	0.297 ± 0.008
Relative	10.61 ± 0.17	10.65 ± 0.18	11.91 ± 0.99	10.55 ± 0.23	11.33 ± 0.49	10.60 ± 0.21
Liver						
Absolute	1.435 ± 0.046	1.511 ± 0.043	1.503 ± 0.053	1.466 ± 0.069	1.580 ± 0.054	1.528 ± 0.046
Relative	49.91 ± 1.60	55.26 ± 1.26*	52.88 ± 1.09	51.24 ± 1.59	53.97 ± 1.21	54.54 ± 1.31
Lung						
Absolute	0.236 ± 0.020	0.219 ± 0.012	0.237 ± 0.014	0.204 ± 0.013	0.207 ± 0.009	0.215 ± 0.011
Relative	8.16 ± 0.63	8.06 ± 0.50	8.39 ± 0.56	7.18 ± 0.47	7.05 ± 0.17	7.69 ± 0.42
R. Testis						
Absolute	0.121 ± 0.003	0.112 ± 0.002**	0.066 ± 0.002**	0.063 ± 0.002**	0.077 ± 0.001**	0.079 ± 0.002**
Relative	4.21 ± 0.05	4.11 ± 0.13	2.34 ± 0.06**	2.22 ± 0.10**	2.63 ± 0.07**	2.82 ± 0.06**
Thymus						
Absolute	0.046 ± 0.003	0.041 ± 0.003	0.042 ± 0.003	0.039 ± 0.003	0.041 ± 0.002	0.040 ± 0.002
Relative	1.63 ± 0.16	1.51 ± 0.11	1.49 ± 0.10	1.36 ± 0.11	1.42 ± 0.09	1.41 ± 0.05

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Study of Phenolphthalein
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Female						
n	10	10	10	10	10	10
Necropsy body wt	23.8 ± 0.7	25.0 ± 0.8	25.3 ± 0.4	25.0 ± 0.5	25.0 ± 0.5	24.8 ± 0.5
Brain						
Absolute	0.474 ± 0.006	0.486 ± 0.006	0.481 ± 0.005	0.493 ± 0.008	0.499 ± 0.008*	0.478 ± 0.004
Relative	20.06 ± 0.49	19.59 ± 0.44	19.08 ± 0.32	19.74 ± 0.38	20.02 ± 0.44	19.31 ± 0.34
Heart						
Absolute	0.127 ± 0.004	0.136 ± 0.003	0.132 ± 0.005	0.132 ± 0.003	0.134 ± 0.004	0.129 ± 0.004
Relative	5.34 ± 0.14	5.48 ± 0.11	5.22 ± 0.14	5.29 ± 0.14	5.37 ± 0.15	5.21 ± 0.18
R. Kidney						
Absolute	0.205 ± 0.005	0.203 ± 0.009	0.221 ± 0.006	0.207 ± 0.004	0.222 ± 0.004	0.218 ± 0.005
Relative	8.68 ± 0.28	8.14 ± 0.21	8.74 ± 0.15	8.27 ± 0.14	8.91 ± 0.17	8.77 ± 0.16
Liver						
Absolute	1.259 ± 0.034	1.287 ± 0.044	1.421 ± 0.035*	1.322 ± 0.049	1.392 ± 0.040	1.396 ± 0.046
Relative	53.12 ± 1.11	51.72 ± 1.46	56.30 ± 1.24	52.81 ± 1.48	55.76 ± 1.18	56.27 ± 1.48
Lung						
Absolute	0.206 ± 0.012	0.209 ± 0.014	0.231 ± 0.020	0.220 ± 0.008	0.214 ± 0.010	0.214 ± 0.013
Relative	8.71 ± 0.51	8.42 ± 0.58	9.10 ± 0.72	8.79 ± 0.31	8.59 ± 0.41	8.60 ± 0.45
Thymus						
Absolute	0.050 ± 0.004	0.056 ± 0.005	0.051 ± 0.003	0.048 ± 0.003	0.053 ± 0.003	0.053 ± 0.003
Relative	2.07 ± 0.12	2.24 ± 0.20	2.03 ± 0.12	1.91 ± 0.12	2.13 ± 0.13	2.15 ± 0.15

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

APPENDIX G
HEMATOLOGY AND CLINICAL CHEMISTRY RESULTS

TABLE G1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of Phenolphthalein[a]

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)						
Day 5	38.3 ± 1.2	40.1 ± 0.9	39.2 ± 0.6	39.4 ± 0.7	38.8 ± 0.9	40.6 ± 0.8
Day 21	43.1 ± 0.8	40.5 ± 1.1	43.1 ± 0.9	44.1 ± 0.7	43.5 ± 0.5	43.1 ± 1.1
Week 13	44.1 ± 0.5	43.2 ± 0.6	44.4 ± 0.4	43.9 ± 0.4	43.3 ± 0.7	43.5 ± 0.6
Hemoglobin (g/dL)						
Day 5	14.5 ± 0.2	14.5 ± 0.2	14.4 ± 0.2	14.4 ± 0.2	14.5 ± 0.2	14.6 ± 0.1
Day 21	15.7 ± 0.1	15.6 ± 0.1	15.7 ± 0.1	15.8 ± 0.1	15.5 ± 0.1	15.6 ± 0.1
Week 13	15.9 ± 0.2	15.5 ± 0.2	16.0 ± 0.4	16.2 ± 0.4	15.5 ± 0.2	15.6 ± 0.2
Erythrocytes ($10^6/\mu L$)						
Day 5	6.50 ± 0.22	6.89 ± 0.17	6.66 ± 0.12	6.56 ± 0.11	6.76 ± 0.14	6.83 ± 0.12
Day 21	7.97 ± 0.17	7.49 ± 0.23	7.89 ± 0.16	8.01 ± 0.14	8.00 ± 0.11	7.78 ± 0.20
Week 13	9.17 ± 0.11	8.90 ± 0.19	9.13 ± 0.10	9.04 ± 0.07	8.84 ± 0.15	8.93 ± 0.13
Reticulocytes ($10^6/\mu L$)						
Day 5	0.25 ± 0.02[b]	0.29 ± 0.03[b]	0.24 ± 0.02[b]	0.23 ± 0.03	0.22 ± 0.02	0.23 ± 0.03[b]
Day 21	0.15 ± 0.02[c]	0.14 ± 0.01[b]	0.15 ± 0.01[d]	0.17 ± 0.01[b]	0.14 ± 0.01[b]	0.16 ± 0.01[c]
Week 13	0.13 ± 0.01[e]	0.14 ± 0.01[f]	0.13 ± 0.01[d]	0.12 ± 0.01[f]	0.11 ± 0.01[f]	0.15 ± 0.01[f]
Mean cell volume (fL)						
Day 5	59.1 ± 1.0	58.1 ± 0.4	59.0 ± 0.6	60.1 ± 0.6	57.4 ± 0.9	59.4 ± 0.9
Day 21	54.2 ± 0.3	54.0 ± 0.3	54.8 ± 0.3	54.8 ± 0.3	54.4 ± 0.4	55.3 ± 0.4
Week 13	48.2 ± 0.3	48.8 ± 0.5	48.4 ± 0.3	48.6 ± 0.3	49.0 ± 0.2	48.7 ± 0.4
Mean cell hemoglobin (pg)						
Day 5	22.5 ± 0.7	21.1 ± 0.4	21.6 ± 0.4	22.0 ± 0.3	21.5 ± 0.3	21.5 ± 0.3
Day 21	19.7 ± 0.4	20.9 ± 0.6	20.0 ± 0.4	19.7 ± 0.2	19.4 ± 0.2	20.2 ± 0.4
Week 13	19.1 ± 1.8	17.4 ± 0.2	17.5 ± 0.4	18.0 ± 0.4	17.6 ± 0.2	17.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 5	38.2 ± 1.2	36.2 ± 0.5	36.8 ± 0.6	36.6 ± 0.5	37.5 ± 0.8	36.2 ± 0.6
Day 21	36.4 ± 0.5	38.7 ± 1.0	36.5 ± 0.8	35.7 ± 0.4	35.7 ± 0.3	36.4 ± 0.7
Week 13	36.1 ± 0.3	35.8 ± 0.3	36.1 ± 0.8	37.0 ± 1.0	35.8 ± 0.3	35.9 ± 0.2
Platelets ($10^3/\mu L$)						
Day 5	998.2 ± 18.5	967.8 ± 18.4	915.8 ± 23.7	898.3 ± 46.3*	907.1 ± 21.7*	916.4 ± 25.2
Day 21	760.7 ± 13.0	814.0 ± 13.3	778.7 ± 8.2	791.8 ± 18.1	817.8 ± 13.3*	848.3 ± 6.9**
Week 13	608.1 ± 11.7	665.3 ± 14.8	645.2 ± 9.7	709.3 ± 22.4**	707.8 ± 62.9*	684.9 ± 11.9**
Leukocytes ($10^3/\mu L$)						
Day 5	6.58 ± 0.28	5.55 ± 0.26	6.08 ± 0.35	6.15 ± 0.45	5.72 ± 0.39	6.73 ± 0.38
Day 21	7.41 ± 0.43	7.22 ± 0.26	7.14 ± 0.20	6.55 ± 0.28	7.03 ± 0.42	7.78 ± 0.31
Week 13	6.03 ± 0.42	6.43 ± 0.38	6.42 ± 0.28	6.98 ± 0.63	6.31 ± 0.38	6.39 ± 0.30
Segmented neutrophils ($10^3/\mu L$)						
Day 5	0.74 ± 0.08	0.81 ± 0.11	0.82 ± 0.11	0.69 ± 0.07	0.60 ± 0.04	0.85 ± 0.08
Day 21	0.95 ± 0.09	1.00 ± 0.12	1.00 ± 0.08	0.81 ± 0.13	0.68 ± 0.08	0.81 ± 0.09
Week 13	1.21 ± 0.07	1.14 ± 0.17	1.08 ± 0.13	1.34 ± 0.16	1.03 ± 0.11	1.19 ± 0.13
Lymphocytes ($10^3/\mu L$)						
Day 5	5.71 ± 0.22	4.58 ± 0.23*	5.07 ± 0.26	5.31 ± 0.46	4.95 ± 0.37	5.73 ± 0.40
Day 21	6.33 ± 0.39	6.16 ± 0.23	6.06 ± 0.23	5.67 ± 0.27	6.26 ± 0.35	6.87 ± 0.26
Week 13	4.63 ± 0.37	5.12 ± 0.25	5.19 ± 0.34	5.44 ± 0.53	5.13 ± 0.33	5.04 ± 0.26

TABLE G1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male (continued)						
n	10	10	10	10	10	10
Hematology (continued)						
Monocytes ($10^3/\mu L$)						
Day 5	0.11 ± 0.04	0.15 ± 0.03	0.19 ± 0.03	0.11 ± 0.03	0.16 ± 0.05	0.15 ± 0.04
Day 21	0.09 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.08 ± 0.01	0.08 ± 0.04	0.09 ± 0.03
Week 13	0.11 ± 0.02	0.14 ± 0.05	0.13 ± 0.02	0.15 ± 0.03	0.11 ± 0.02	0.11 ± 0.04
Eosinophils ($10^3/\mu L$)						
Day 5	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.01 ± 0.01
Day 21	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Week 13	0.08 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	0.09 ± 0.01
n	10	10	10	10	10	10
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 5	19.0 ± 0.6	18.8 ± 0.5	18.3 ± 0.4	17.5 ± 0.7	18.3 ± 0.4	18.0 ± 0.6
Day 21	20.8 ± 0.4	20.9 ± 0.3	21.5 ± 0.4	21.9 ± 0.5	21.9 ± 1.1	22.8 ± 0.6
Week 13	20.3 ± 0.5	20.1 ± 0.6	19.6 ± 0.8	21.4 ± 0.6	19.7 ± 0.9	21.0 ± 0.7
Creatinine (mg/dL)						
Day 5	0.60 ± 0.03	0.62 ± 0.03	0.60 ± 0.02	0.57 ± 0.03	0.55 ± 0.02	0.55 ± 0.02
Day 21	0.64 ± 0.03	0.56 ± 0.05	0.58 ± 0.03	0.61 ± 0.02	0.59 ± 0.03[b]	0.58 ± 0.02
Week 13	0.81 ± 0.07	0.72 ± 0.05	0.66 ± 0.04	0.60 ± 0.02**	0.65 ± 0.03	0.64 ± 0.03*
Total protein (g/dL)						
Day 5	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
Day 21	7.1 ± 0.1	7.1 ± 0.1	7.2 ± 0.2	7.4 ± 0.1	7.3 ± 0.1	7.5 ± 0.1
Week 13	7.5 ± 0.3	7.6 ± 0.2	7.6 ± 0.1	7.9 ± 0.1	8.2 ± 0.2	7.9 ± 0.3
Albumin (g/dL)						
Day 5	4.2 ± 0.2	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
Day 21	4.5 ± 0.0	4.5 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.7 ± 0.1
Week 13	4.7 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.1
Alanine aminotransferase (IU/L)						
Day 5	32 ± 1	30 ± 1	31 ± 1	34 ± 1	33 ± 1	39 ± 1*
Day 21	37 ± 2	41 ± 3	37 ± 2	37 ± 1	39 ± 2	42 ± 2
Week 13	45 ± 2	37 ± 2	36 ± 2	43 ± 3	33 ± 2**	43 ± 6
Alkaline phosphatase (IU/L)						
Day 5	541 ± 9	565 ± 14	550 ± 6	556 ± 9	535 ± 12	557 ± 13
Day 21	376 ± 10	379 ± 16	379 ± 5	386 ± 7[b]	355 ± 11	366 ± 9
Week 13	185 ± 7	173 ± 5	184 ± 10	181 ± 4	184 ± 9	188 ± 6
Creatine kinase (IU/L)						
Day 5	178 ± 25	228 ± 51	216 ± 53	319 ± 83	197 ± 59	177 ± 35
Day 21	152 ± 18	172 ± 30	139 ± 14	152 ± 15[b]	158 ± 21	132 ± 9
Week 13	212 ± 40	134 ± 17	148 ± 19	223 ± 43	172 ± 20	168 ± 30
Sorbitol dehydrogenase (IU/L)						
Day 5	8 ± 1	9 ± 1	9 ± 1	11 ± 1	7 ± 1	7 ± 1
Day 21	14 ± 2	9 ± 1	10 ± 1	11 ± 1	10 ± 1	9 ± 1*
Week 13	8 ± 1	8 ± 1	9 ± 1	10 ± 1	8 ± 1	11 ± 2
Bile acids ($\mu mol/L$)						
Day 5	25.2 ± 4.1	17.5 ± 3.7	8.3 ± 0.7**	9.2 ± 2.0**	10.0 ± 1.1	8.9 ± 0.9**
Day 21	8.9 ± 1.3	5.3 ± 0.6*	5.3 ± 0.4*	9.1 ± 3.2[b]	5.0 ± 0.8*[b]	6.2 ± 0.3[b]
Week 13	20.8 ± 8.3	18.6 ± 8.7	14.8 ± 6.9	19.9 ± 7.4[b]	23.0 ± 8.4	19.5 ± 9.6

TABLE G1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Female						
n	10	10	10	9	10	10
Hematology						
Hematocrit (%)						
Day 5	39.8 ± 1.0	39.6 ± 0.7	39.6 ± 0.6	40.2 ± 1.2	39.8 ± 0.9	39.6 ± 1.1
Day 21	41.3 ± 0.5	43.5 ± 0.6	42.5 ± 0.7	42.2 ± 1.2	43.0 ± 0.6	42.8 ± 0.7
Week 13	44.1 ± 0.3	44.1 ± 0.3	43.7 ± 0.6	43.0 ± 0.8	43.6 ± 0.5	44.3 ± 0.4
Hemoglobin (g/dL)						
Day 5	15.1 ± 0.2	15.1 ± 0.2	15.2 ± 0.1	15.1 ± 0.2	15.2 ± 0.2	15.0 ± 0.2
Day 21	15.9 ± 0.2	15.9 ± 0.1	16.1 ± 0.2	15.8 ± 0.2	16.0 ± 0.1	15.6 ± 0.1
Week 13	16.3 ± 0.2	15.9 ± 0.2	16.1 ± 0.2	16.2 ± 0.2	16.1 ± 0.3	16.6 ± 0.4
Erythrocytes ($10^6/\mu L$)						
Day 5	7.05 ± 0.23	7.01 ± 0.13	7.00 ± 0.11	7.08 ± 0.23	7.12 ± 0.19	6.98 ± 0.18
Day 21	7.64 ± 0.09	8.02 ± 0.11	7.81 ± 0.16	7.75 ± 0.23	7.90 ± 0.12	7.89 ± 0.11
Week 13	8.61 ± 0.08	8.60 ± 0.07	8.54 ± 0.12	8.36 ± 0.18	8.50 ± 0.12	8.61 ± 0.08
Reticulocytes ($10^6/\mu L$)						
Day 5	0.19 ± 0.02	0.19 ± 0.01	0.18 ± 0.02[b]	0.16 ± 0.01[c]	0.16 ± 0.01[b]	0.14 ± 0.02[c]
Day 21	0.12 ± 0.01[c]	0.14 ± 0.01[c]	0.13 ± 0.01[c]	0.13 ± 0.01[d]	0.14 ± 0.01[c]	0.13 ± 0.01[c]
Week 13	0.13 ± 0.01[f]	0.12 ± 0.01[f]	0.12 ± 0.01[e]	0.12 ± 0.01[g]	0.12[h]	0.12 ± 0.00[f]
Mean cell volume (eL)						
Day 5	56.7 ± 0.6	56.5 ± 0.2	56.9 ± 0.7	56.8 ± 0.4	56.0 ± 0.3	56.6 ± 0.7
Day 21	54.2 ± 0.3	54.2 ± 0.3	54.4 ± 0.4	54.4 ± 0.3	54.2 ± 0.3	54.4 ± 0.3
Week 13	51.1 ± 0.2	51.2 ± 0.3	51.0 ± 0.3	51.4 ± 0.2	51.2 ± 0.2	51.3 ± 0.2
Mean cell hemoglobin (pg)						
Day 5	21.6 ± 0.6	21.5 ± 0.3	21.8 ± 0.3	21.4 ± 0.7	21.4 ± 0.4	21.6 ± 0.4
Day 21	20.8 ± 0.3	19.9 ± 0.3	20.7 ± 0.3	20.6 ± 0.7	20.3 ± 0.3	19.8 ± 0.3
Week 13	18.9 ± 0.2	18.5 ± 0.1	18.8 ± 0.1	19.4 ± 0.5	18.9 ± 0.3	19.2 ± 0.4
Mean cell hemoglobin concentration (g/dL)						
Day 5	38.1 ± 0.8	38.1 ± 0.5	38.5 ± 0.4	37.7 ± 1.1	38.2 ± 0.6	38.0 ± 0.8
Day 21	38.5 ± 0.5	36.6 ± 0.5*	38.0 ± 0.4	37.8 ± 1.2	37.3 ± 0.5	36.5 ± 0.5*
Week 13	36.9 ± 0.4	36.0 ± 0.3	36.7 ± 0.2	37.7 ± 0.9	37.0 ± 0.6	37.4 ± 0.9
Platelets ($10^3/\mu L$)						
Day 5	856.0 ± 18.1	863.0 ± 12.2	870.5 ± 13.4	867.0 ± 11.7	841.0 ± 16.2	823.1 ± 17.6
Day 21	721.3 ± 10.2	748.2 ± 15.2	711.3 ± 26.3	741.7 ± 12.9	746.5 ± 8.5	748.4 ± 13.6
Week 13	638.7 ± 14.6	652.1 ± 10.0	673.2 ± 9.5	672.2 ± 8.6	656.6 ± 14.4	673.1 ± 14.2
Leukocytes ($10^3/\mu L$)						
Day 5	6.14 ± 0.21	6.17 ± 0.30	6.54 ± 0.22	5.75 ± 0.46[c]	6.07 ± 0.18	5.91 ± 0.29
Day 21	6.11 ± 0.20	6.21 ± 0.18	6.70 ± 0.28	6.53 ± 0.44[c]	7.49 ± 0.26**	6.92 ± 0.40
Week 13	5.40 ± 0.33	5.52 ± 0.22	5.46 ± 0.31	6.18 ± 0.27[c]	5.44 ± 0.26	5.68 ± 0.42
Segmented neutrophils ($10^3/\mu L$)						
Day 5	0.67 ± 0.05	0.68 ± 0.07	0.65 ± 0.06	0.61 ± 0.06[c]	0.69 ± 0.07	0.72 ± 0.09
Day 21	0.57 ± 0.03	0.72 ± 0.06	0.78 ± 0.13	0.58 ± 0.08[c]	0.93 ± 0.07**	0.70 ± 0.08
Week 13	1.04 ± 0.15	0.87 ± 0.11	1.11 ± 0.16	0.78 ± 0.08[c]	1.07 ± 0.14	0.88 ± 0.10
Lymphocytes ($10^3/\mu L$)						
Day 5	5.34 ± 0.19	5.24 ± 0.28	5.70 ± 0.16	4.99 ± 0.40[c]	5.25 ± 0.20	5.05 ± 0.28
Day 21	5.43 ± 0.20	5.44 ± 0.20	5.80 ± 0.22	5.85 ± 0.41[c]	6.47 ± 0.23*	6.14 ± 0.34
Week 13	4.19 ± 0.29	4.53 ± 0.20	4.21 ± 0.26	5.30 ± 0.27[c]	4.21 ± 0.22	4.66 ± 0.37

TABLE G1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Female (continued)						
n	10	10	10	9	10	10
Hematology (continued)						
Monocytes ($10^3/\mu L$)						
Day 5	0.12 ± 0.03	0.18 ± 0.04	0.16 ± 0.04	0.14 ± 0.04[c]	0.11 ± 0.03	0.07 ± 0.02
Day 21	0.09 ± 0.03	0.08 ± 0.02	0.10 ± 0.02	0.09 ± 0.02[c]	0.09 ± 0.03	0.09 ± 0.03
Week 13	0.13 ± 0.04	0.09 ± 0.03	0.11 ± 0.03	0.10 ± 0.03[c]	0.12 ± 0.04	0.11 ± 0.03
Eosinophils ($10^3/\mu L$)						
Day 5	0.04 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.03 ± 0.02[c]	0.03 ± 0.02	0.07 ± 0.02
Day 21	0.03 ± 0.02	0.01 ± 0.01	0.04 ± 0.02	0.05 ± 0.02[c]	0.01 ± 0.01	0.01 ± 0.01
Week 13	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	0.03 ± 0.02[c]	0.03 ± 0.02	0.04 ± 0.02
n						
Day 5	10	10	9	9	10	10
Day 21	10	10	10	9	10	10
Week 13	10	10	10	9	10	10
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 5	21.1 ± 0.7	18.6 ± 0.5	19.9 ± 0.5	18.5 ± 0.6*	20.0 ± 1.2	18.2 ± 0.5**
Day 21	21.5 ± 0.7	20.4 ± 0.6	20.4 ± 0.5	20.8 ± 0.5	22.0 ± 0.5	21.3 ± 0.5
Week 13	19.0 ± 0.8	20.5 ± 0.9	19.7 ± 0.9	20.1 ± 1.1	20.2 ± 0.7	19.5 ± 0.8
Creatinine (mg/dL)						
Day 5	0.55 ± 0.01	0.56 ± 0.02	0.62 ± 0.02	0.55 ± 0.03	0.57 ± 0.02	0.54 ± 0.02
Day 21	0.58 ± 0.03	0.61 ± 0.04	0.64 ± 0.03	0.60 ± 0.02	0.61 ± 0.03	0.60 ± 0.03
Week 13	0.71 ± 0.02	0.70 ± 0.02	0.74 ± 0.02	0.68 ± 0.03	0.70 ± 0.02	0.67 ± 0.02
Total protein (g/dL)						
Day 5	6.3 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.1 ± 0.1
Day 21	7.0 ± 0.1	7.0 ± 0.1	7.2 ± 0.2	7.0 ± 0.1	7.1 ± 0.1	6.9 ± 0.1[b]
Week 13	8.0 ± 0.1	8.0 ± 0.2	7.8 ± 0.2	7.6 ± 0.2	8.1 ± 0.1	8.1 ± 0.2
Albumin (g/dL)						
Day 5	4.6 ± 0.1	4.4 ± 0.1*	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.0	4.3 ± 0.0*
Day 21	4.6 ± 0.1	4.5 ± 0.0	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.5 ± 0.1
Week 13	5.0 ± 0.1	5.0 ± 0.1	4.8 ± 0.2	4.9 ± 0.1	5.1 ± 0.1	5.0 ± 0.1
Alanine aminotransferase (IU/L)						
Day 5	30 ± 1	26 ± 1	29 ± 1	26 ± 2	31 ± 1	29 ± 1
Day 21	33 ± 1	33 ± 1[b]	32 ± 2	30 ± 1	33 ± 1	35 ± 1
Week 13	37 ± 2	36 ± 1	37 ± 2	28 ± 2	29 ± 1	28 ± 1*
Alkaline phosphatase (IU/L)						
Day 5	441 ± 11	420 ± 9	428 ± 11	420 ± 12	432 ± 14	405 ± 9
Day 21	294 ± 9	294 ± 14	300 ± 12	294 ± 11	295 ± 6	293 ± 6
Week 13	159 ± 7	160 ± 5	159 ± 7	148 ± 8	162 ± 6	148 ± 6
Creatine kinase (IU/L)						
Day 5	153 ± 16	142 ± 11	216 ± 74	154 ± 38	168 ± 24	114 ± 11
Day 21	219 ± 64	153 ± 16	163 ± 22	152 ± 39	127 ± 11	117 ± 11
Week 13	169 ± 20	136 ± 23	113 ± 14	215 ± 84	171 ± 20	198 ± 64

TABLE G1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Feed Study of Phenolphthalein (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Female (continued)						
n						
Day 5	10	10	9	9	10	10
Day 21	10	10	10	9	10	10
Week 13	10	10	10	9	10	10
Clinical Chemistry (continued)						
Sorbitol dehydrogenase (IU/L)						
Day 5	6 ± 1	6 ± 0	7 ± 1	7 ± 1	7 ± 1	6 ± 1
Day 21	9 ± 2	12 ± 2	12 ± 2	11 ± 2	11 ± 1	12 ± 1
Week 13	8 ± 1	7 ± 1	8 ± 0	7 ± 1	7 ± 1	7 ± 0
Bile acids (μmol/L)						
Day 5	21.3 ± 4.9	11.9 ± 1.3	17.3 ± 3.8	9.3 ± 1.3*	13.9 ± 2.1	10.4 ± 2.2
Day 21	11.8 ± 2.4[b]	7.5 ± 1.0	9.7 ± 1.2	8.6 ± 1.5	9.4 ± 0.7	8.8 ± 1.0
Week 13	23.5 ± 5.2	20.0 ± 3.7	20.9 ± 6.9	15.9 ± 5.6	21.6 ± 9.1	23.6 ± 7.9

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=8
[d] n=7
[e] n=5
[f] n=3
[g] n=4
[h] n=1; no standard error calculated

APPENDIX H
DETERMINATIONS OF TOTAL PHENOLPHTHALEIN IN PLASMA

TABLE H1
Plasma Concentrations and Estimated Area Under the Curve Values of Total Phenolphthalein in Rats in the 2-Year Feed Study of Phenolphthalein[a]

	12,000 ppm	25,000 ppm	50,000 ppm
n	3	3	3
Male			
Plasma Concentrations[b]			
6 a.m.	107.80 ± 32.81[c]	127.00 ± 72.58	177.23 ± 185.33
9 a.m.	102.43 ± 8.79	63.50 ± 50.79	73.20 ± 24.91
1 p.m.	59.63 ± 2.54	34.45 ± 15.77[c]	157.47 ± 181.13
4 p.m.	57.40 ± 9.93	101.23 ± 86.08	224.40 ± 232.78[c]
9 p.m.	69.17 ± 10.18	59.53 ± 16.70	80.85 ± 13.08[c]
Area Under the Curve			
6 a.m.-9 p.m.	1,131 ± 49	1,087 ± 217	2,173 ± 587
Female			
Plasma Concentrations			
6 a.m.	94.13 ± 25.01	90.10 ± 12.05	104.23 ± 32.87
9 a.m.	92.77 ± 23.48	110.10 ± 41.79	108.33 ± 3.51
1 p.m.	68.17 ± 9.92	101.37 ± 13.45	104.07 ± 34.28
4 p.m.	97.50 ± 32.15	72.83 ± 23.25	78.37 ± 17.45
9 p.m.	69.23 ± 12.96	110.77 ± 40.25	83.30 ± 10.38
Area Under the Curve			
6 a.m.-9 p.m.	1,268 ± 94	1,444 ± 125	1,422 ± 81

[a] Data are presented as mean ± standard deviation. Plasma concentrations are given in μg/mL. Area under the curve values are given as μg•hour/mL. Total phenolphthalein equals free and conjugated phenolphthalein.
[b] Samples were collected on the last day of the study.
[c] n=2

TABLE H2
Plasma Concentrations and Estimated Area Under the Curve Values of Total Phenolphthalein in Mice in the 2-Year Feed Study of Phenolphthalein[a]

	3,000 ppm	6,000 ppm	12,000 ppm
n	3	3	3
Male			
Plasma Concentrations[b]			
6 a.m.	128.67 ± 13.50	149.00 ± 33.15	174.0 ± 9.0
9 a.m.	100.47 ± 18.49	112.00 ± 20.95	159.0 ± 57.5
1 p.m.	177.00 ± 90.54	106.43 ± 13.98	105.3 ± 17.3
4 p.m.	99.67 ± 20.98	121.17 ± 35.75	109.8 ± 38.1
9 p.m.	102.03 ± 6.94	121.33 ± 15.88	175.7 ± 11.4
Area Under the Curve			
6 a.m.-9 p.m.	1,818 ± 166	1,776 ± 106	2,065 ± 135
Female			
Plasma Concentrations			
6 a.m.	112.00 ± 57.00	180.63 ± 17.86	233.13 ± 34.49
9 a.m.	130.57 ± 10.85	169.77 ± 15.77	205.40 ± 26.61
1 p.m.	127.40 ± 32.08	135.93 ± 14.59	149.93 ± 3.90
4 p.m.	101.93 ± 8.80	110.97 ± 14.62	175.60 ± 31.13
9 p.m.	134.53 ± 32.10	112.27 ± 48.05	203.27 ± 114.67
Area Under the Curve			
6 a.m.-9 p.m.	1,815 ± 103	2,066 ± 104	2,804 ± 222

[a] Data are presented as mean ± standard deviation. Plasma concentrations are given in μg/mL. Area under the curve values are given as μg•hour/mL. Total phenolphthalein equals free and conjugated phenolphthalein.
[b] Samples were collected on the last day of the study.

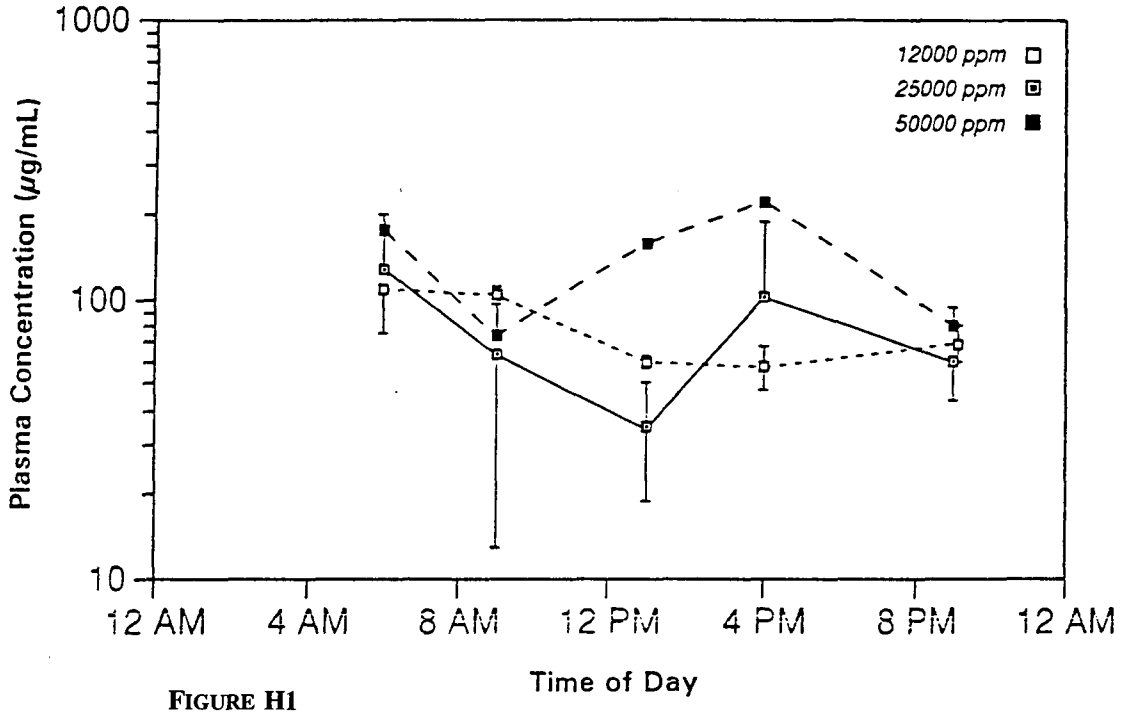

FIGURE H1
Plasma Concentrations of Total Phenolphthalein in Male Rats After Exposure to 12,000, 25,000, or 50,000 ppm in Feed for 2 Years

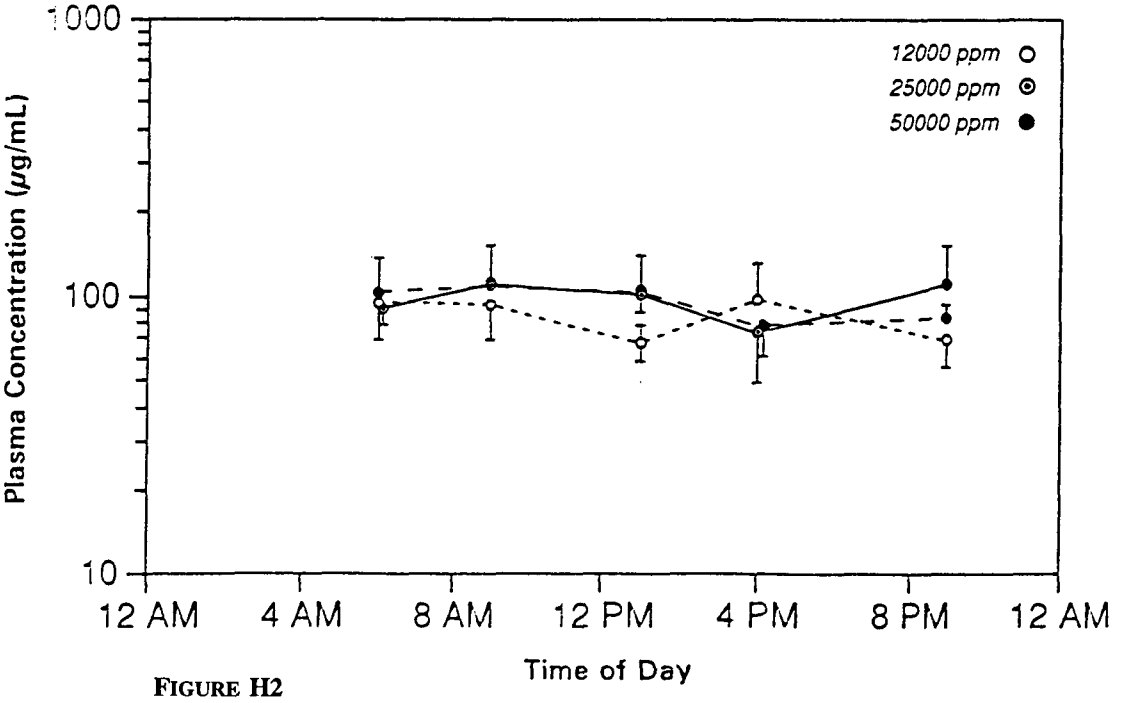

FIGURE H2
Plasma Concentrations of Total Phenolphthalein in Female Rats After Exposure to 12,000, 25,000, or 50,000 ppm in Feed for 2 Years

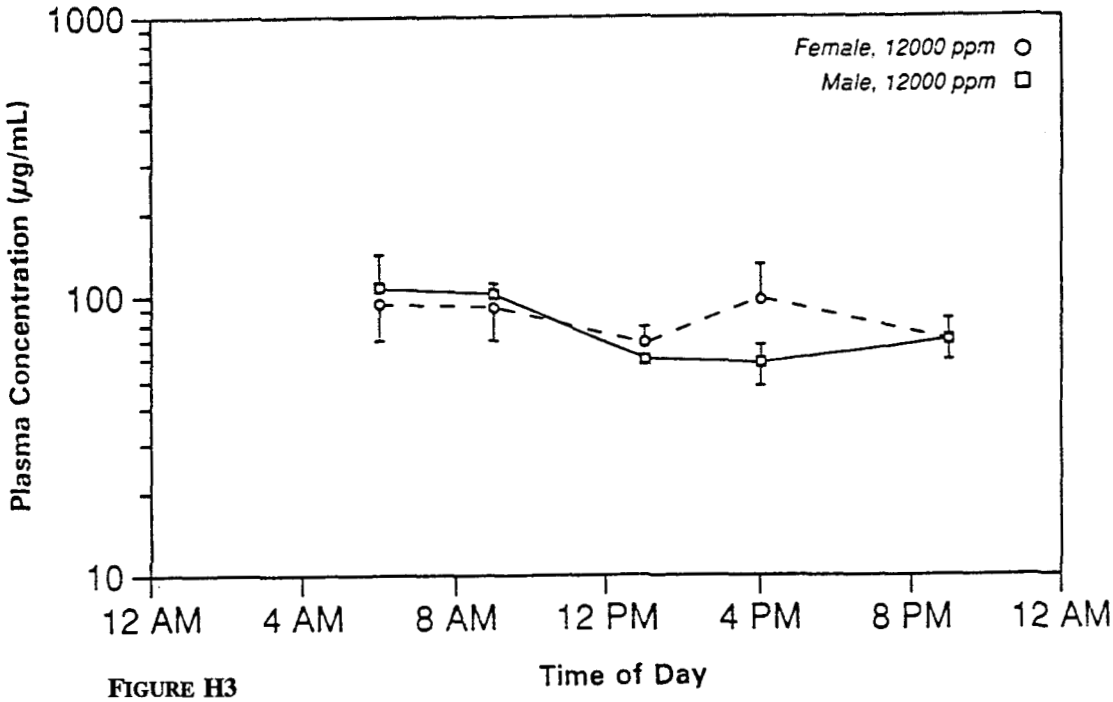

FIGURE H3
Plasma Concentrations of Total Phenolphthalein in Male and Female Rats After Exposure to 12,000 ppm in Feed for 2 Years

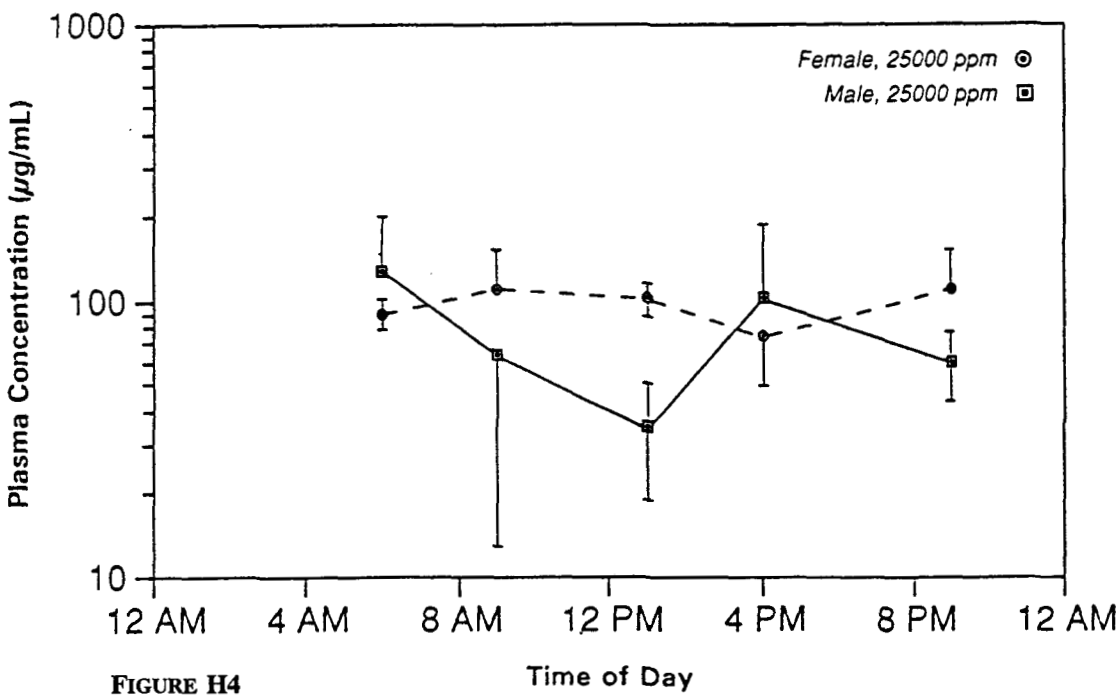

FIGURE H4
Plasma Concentrations of Total Phenolphthalein in Male and Female Rats After Exposure to 25,000 ppm in Feed for 2 Years

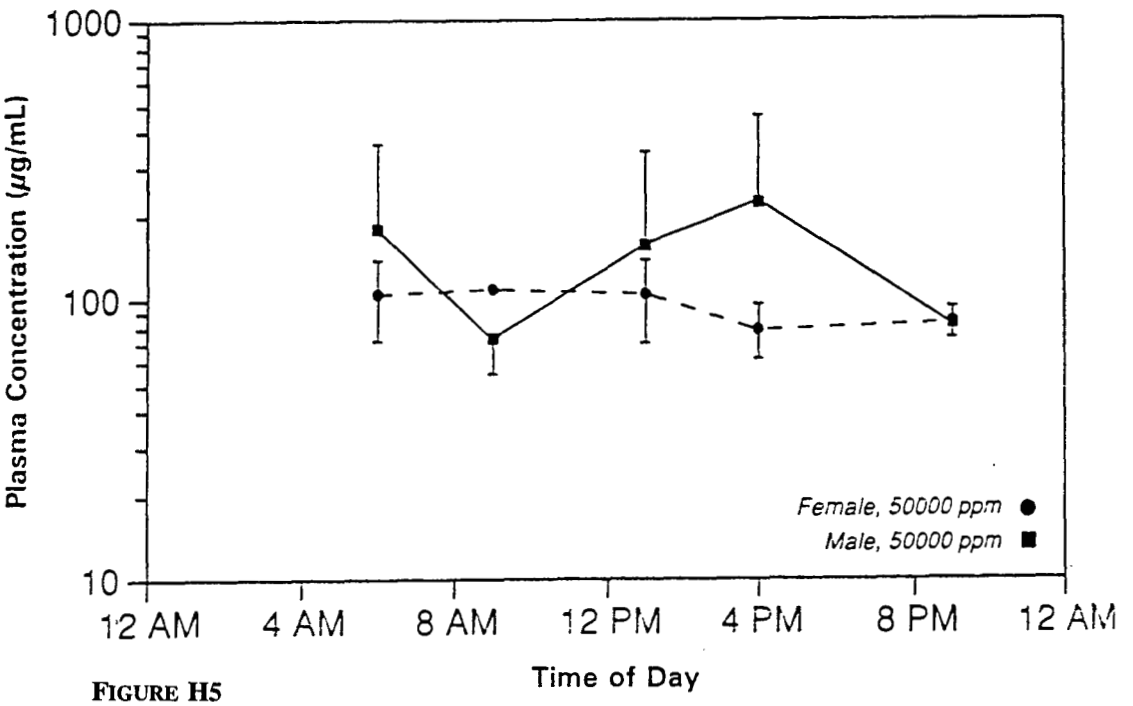

FIGURE H5
Plasma Concentrations of Total Phenolphthalein in Male and Female Rats After Exposure to 50,000 ppm in Feed for 2 Years

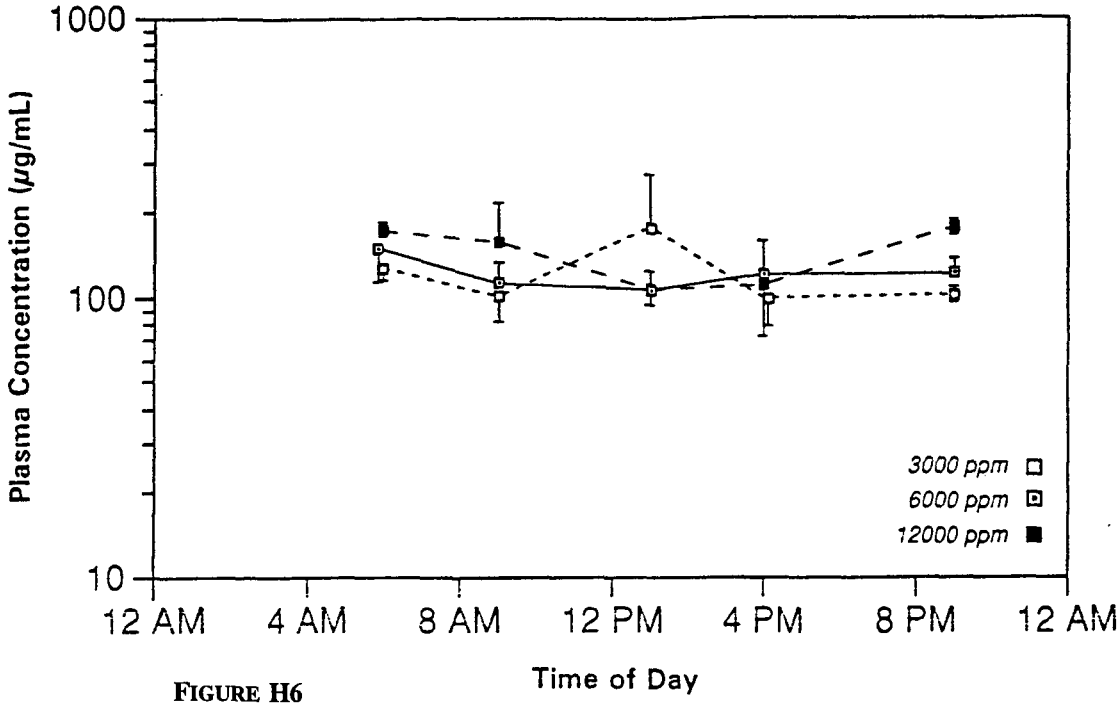

FIGURE H6
Plasma Concentrations of Total Phenolphthalein in Male Mice After Exposure to 3,000, 6,000, or 12,000 ppm in Feed for 2 Years

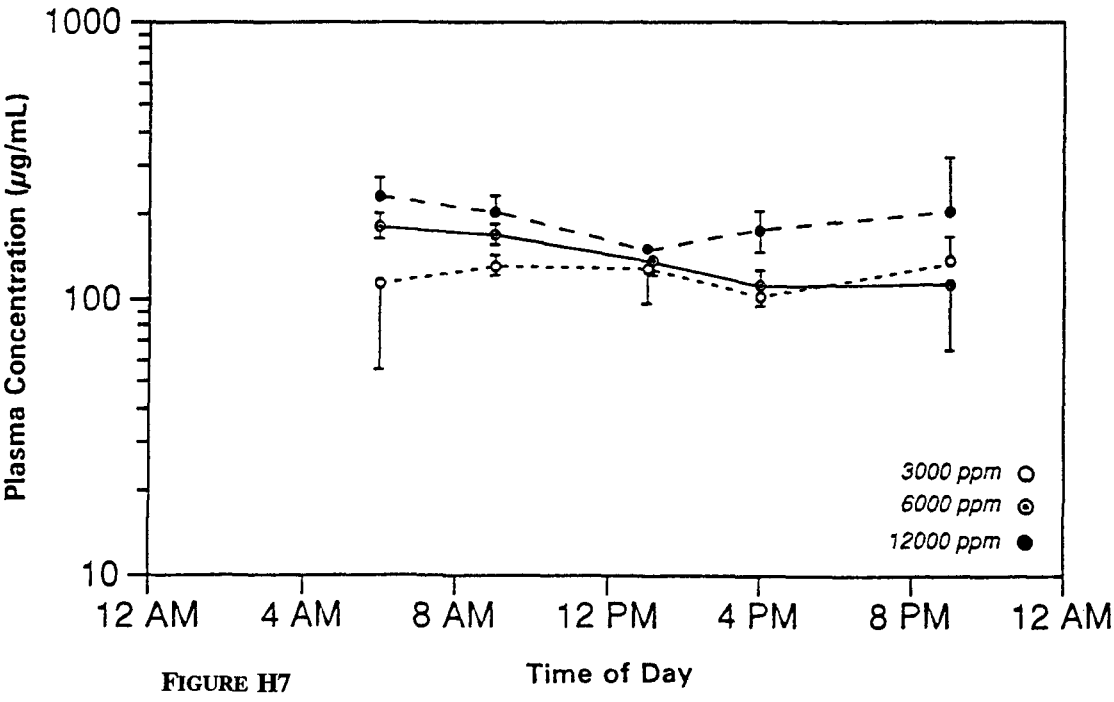

FIGURE H7
Plasma Concentrations of Total Phenolphthalein in Female Mice After Exposure to 3,000, 6,000, or 12,000 ppm in Feed for 2 Years

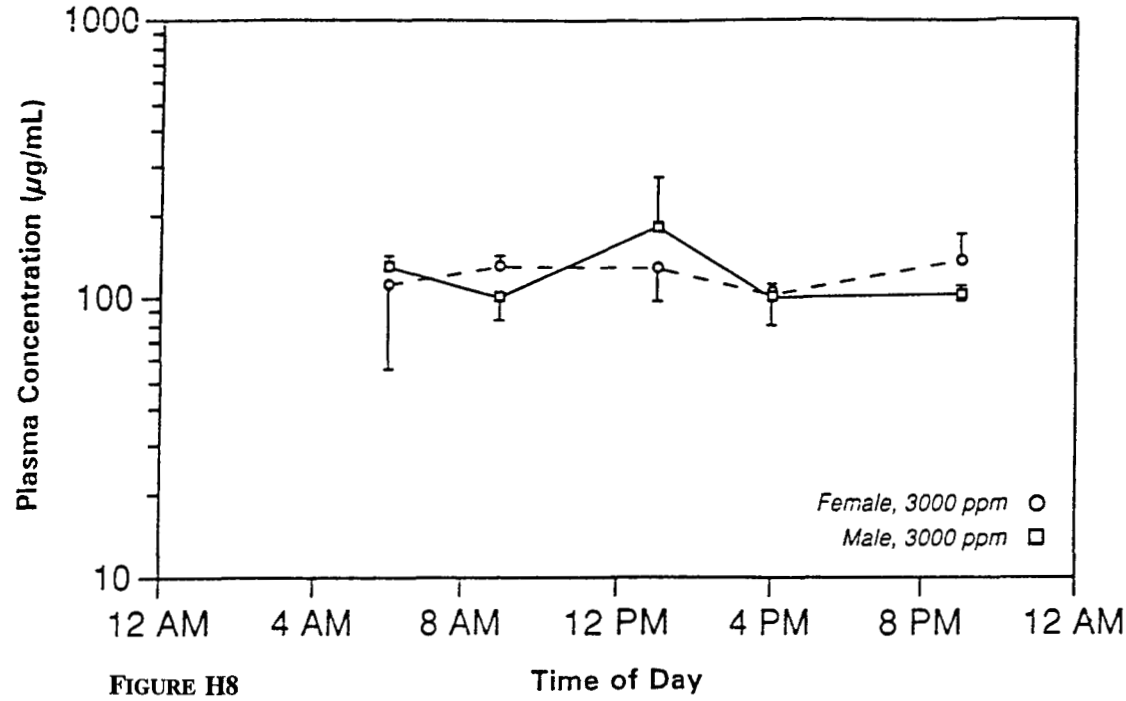

FIGURE H8
Plasma Concentrations of Total Phenolphthalein in Male and Female Mice After Exposure to 3,000 ppm in Feed for 2 Years

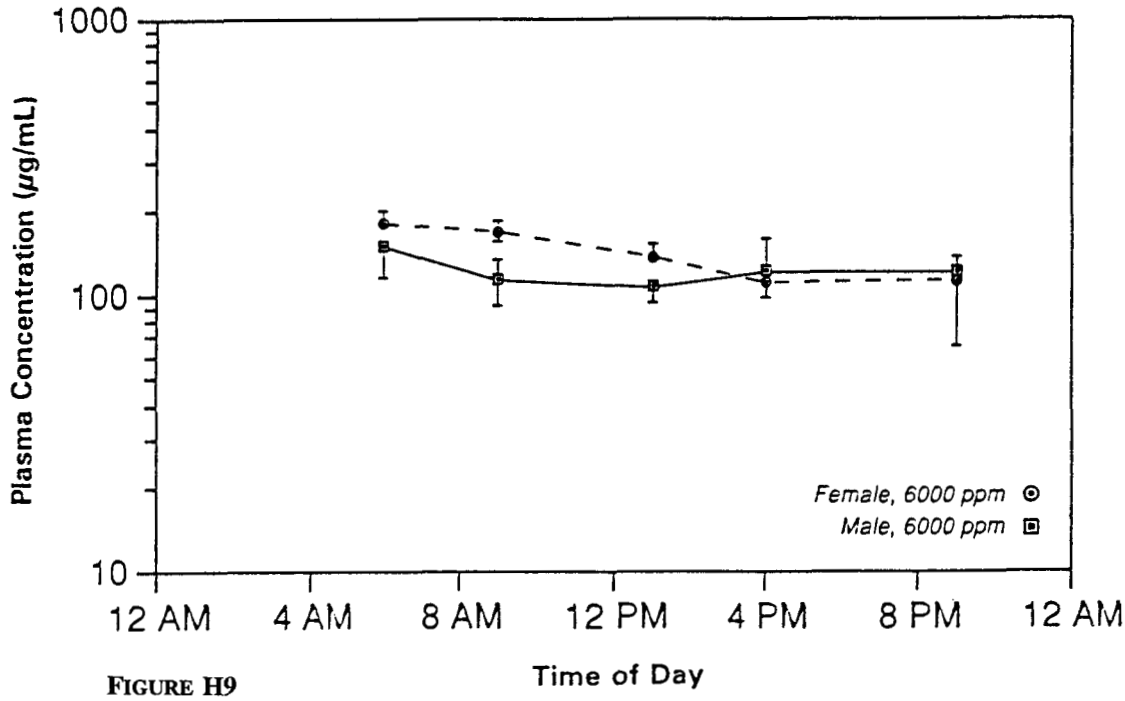

FIGURE H9
Plasma Concentrations of Total Phenolphthalein in Male and Female Mice After Exposure to 6,000 ppm in Feed for 2 Years

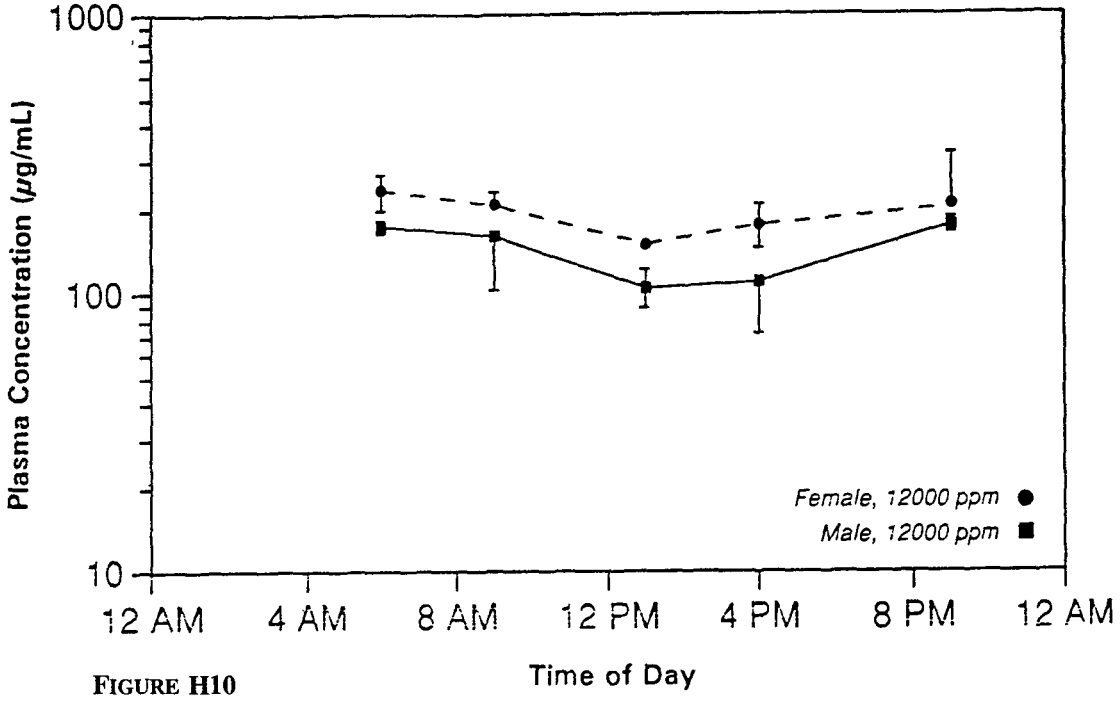

FIGURE H10
Plasma Concentrations of Total Phenolphthalein in Male and Female Mice After Exposure to 12,000 ppm in Feed for 2 Years

APPENDIX I
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE I1
Summary of Reproductive Tissue Evaluations and Estrous Cycle Characterization for Rats in the 13-Week Feed Study of Phenolphthalein[a]

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male				
n	10	10	10	10
Weights (g)				
Necropsy body wt	360 ± 6	356 ± 6	359 ± 10	352 ± 6
R. cauda	0.194 ± 0.006	0.198 ± 0.006	0.203 ± 0.005	0.207 ± 0.005
R. epididymis	0.448 ± 0.007	0.454 ± 0.009	0.458 ± 0.009	0.452 ± 0.009
R. testis	1.481 ± 0.029	1.502 ± 0.030	1.525 ± 0.042	1.517 ± 0.029
Epididymal spermatozoal parameters				
Motility (%)	61.60 ± 2.06	72.35 ± 2.20*	67.19 ± 2.49	67.84 ± 4.23
Abnormal (%)	0.680 ± 0.134	0.760 ± 0.107	0.840 ± 0.126	0.800 ± 0.107
Concentration (10^6/g cauda epididymal tissue)	368 ± 14	352 ± 32	381 ± 18	386 ± 22
Female				
n	10	10	10	10
Necropsy body wt (g)	210 ± 3	205 ± 3	202 ± 3	198 ± 3*
Estrous cycle length (days)	4.90 ± 0.10	4.60 ± 0.16	4.80 ± 0.20	4.70 ± 0.15
Estrous stages (% of cycle)				
Diestrus	40.0	38.6	34.3	40.0
Proestrus	18.6	20.0	14.3	20.0
Estrus	17.1	21.4	28.6	18.6
Metestrus	24.3	20.0	22.9	21.4

* Significantly different ($P \leq 0.05$) from the controls by Dunnett's test (body weight) or by Dunn's test (motility)

[a] Weights, epididymal spermatozoal parameters, and estrous cycle length are presented as mean ± standard error. Differences from the control group for organ weights and estrous cycle length are not significant by Dunn's or Dunnett's test. By multivariate analysis of variance, exposed females did not differ significantly from the control females in relative length of time spent in the estrous stages.

TABLE I2
Summary of Reproductive Tissue Evaluations and Estrous Cycle Characterization for Mice in the 13-Week Feed Study of Phenolphthalein[a]

	0 ppm	12,000 ppm	25,000 ppm	50,000 ppm
Male				
n	10	10	10	10
Weights (g)				
Necropsy body wt	28.8 ± 0.8	28.5 ± 0.6	29.3 ± 0.8	28.0 ± 0.6
R. cauda	0.022 ± 0.001	0.018 ± 0.001*	0.019 ± 0.001	0.019 ± 0.001
R. epididymis	0.049 ± 0.002	0.038 ± 0.001**	0.040 ± 0.001**	0.040 ± 0.001**
R. testis	0.121 ± 0.003	0.063 ± 0.002**	0.077 ± 0.001**	0.079 ± 0.002**
Epididymal spermatozoal parameters				
Motility (%)	78.88 ± 1.95	79.22 ± 1.90	79.83 ± 1.74	75.37 ± 1.71
Abnormal (%)	1.30 ± 0.10	5.74 ± 0.63**	4.80 ± 0.45**	5.74 ± 0.26**
Concentration (10^6/g cauda epididymal tissue)	590 ± 54	349 ± 33*	417 ± 50	288 ± 32**
Female				
n	10	10	10	10
Necropsy body wt (g)	23.8 ± 0.7	25.0 ± 0.5	25.0 ± 0.5	24.8 ± 0.5
Estrous cycle length (days)	4.00 ± 0.00	4.56 ± 0.29[b]	4.20 ± 0.20[c]	4.25 ± 0.16[d]
Estrous stages (% of cycle)				
Diestrus	37.1	31.4	38.6	27.1
Proestrus	25.7	21.4	15.7	15.7
Estrus	28.6	34.3	34.3	41.1
Metestrus	8.6	12.9	11.4	15.7

* Significantly different ($P \leq 0.05$) from the controls by Williams' or Dunnett's test (weights) or by Dunn's test (epididymal spermatozoal parameters)
** $P \leq 0.01$
[a] Weights, epididymal spermatozoal parameters, and estrous cycle length are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's or Dunnett's test. By multivariate analysis of variance, exposed females did not differ significantly from the control females in relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 7 days or was unclear in 1 of 10 animals.
[c] Estrous cycle was longer than 7 days or was unclear in 5 of 10 animals.
[d] Estrous cycle was longer than 7 days or was unclear in 2 of 10 animals.

APPENDIX J
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF PHENOLPHTHALEIN

Phenolphthalein was obtained from Air Products and Chemicals, Inc. (Allentown, PA), in one lot (127-7809) and from Pharmco Laboratories, Inc. (Titusville, FL), in two lots (P3186-D5 and P9189-J1). Lot 127-7809 was used during the 14-day studies, lot P3186-D5 was used during the 13-week studies, and lot P9189-J1 was used during the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). Reports on analyses performed in support of the phenolphthalein studies are on file at the National Institute of Environmental Health Sciences.

All lots of the chemical, a yellow powder, were identified as phenolphthalein by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy and by melting point. All spectra were consistent with the literature spectra (*Sadtler Standard Spectra*) of phenolphthalein. The infrared and nuclear magnetic resonance spectra are presented in Figures J1 and J2. The melting points of all lots of the chemical were consistent with literature values (*Merck Index*, 1989).

The purity of each lot was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). For functional group titration of lot 127-7809, samples were titrated with 0.1 N tetrabutylammonium hydroxide in 2-propanol. For functional group titration of lots P3186-D5 and P9189-J1, samples were dissolved in dimethylformamide and reacted with excess iodine. The unreacted iodine was titrated with standardized 0.1 N sodium thiosulfate to a colorimetric endpoint using a starch indicator. TLC was performed on Silica Gel 60 F-254 plates for lots 127-7809 and P3186-D5 and on Whatman Silica Gel 60A K6F plates for lot P9189-J1. Three solvent systems were used: 1) carbon tetrachloride:diethyl ether:95% ethanol (53:42:5), 2) chloroform:ethyl acetate (80:20), and 3) acetone:methylene chloride (80:20). System 1 was used for all lots, system 2 was used for lot 127-7809, and system 3 was used for lots P3186-D5 and P9189-J1. For all lots, plates were examined under ultraviolet light (254 and/or 366 nm) and with a spray of 1 N aqueous sodium hydroxide. *o*-Cresolphthalein (10 μg/μL in methanol) was used as a reference standard. For lot P3186-D5, plates were also examined under visible light. HPLC for all lots was performed with a Waters μBondapak C_{18} column using ultraviolet detection (280 nm) and a flow rate 1.0 mL/minute. Mobile phases of water:methanol (45:55 and 55:45) were used for lot 127-7809. Mobile phases of water with 1% glacial acetic acid:methanol with 1% glacial acetic acid with ratios of 50:50 and 54:46 were used for lots P3186-D5 and P9189-J1, respectively.

For lot 127-7809, elemental analyses for carbon, hydrogen, and oxygen were in agreement with the theoretical values for phenolphthalein. Karl Fischer water analysis indicated 0.36% $\pm$ 0.02% water. Functional group titration indicated a purity of 98.8% $\pm$ 0.8%. TLC by systems 1 and 2 indicated a major spot, a minor impurity, a trace impurity, and a slight trace impurity. HPLC with a mobile phase ratio of 45:55 revealed a major peak and five impurities with a combined area of 2.19% relative to the major peak area. HPLC with a mobile phase ratio of 55:45 revealed a major peak and four impurities with a combined area of 2.28% of the major peak area. The overall purity of lot 127-7809 was determined to be greater than or equal to 98%.

For lot P3186-D5, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for phenolphthalein. Karl Fischer water analysis indicated 0.193% $\pm$ 0.004% water. Functional group titration indicated a purity of 99.5% $\pm$ 0.4%. TLC indicated a major spot, a minor impurity, and a trace impurity by system 1 and a major spot and a trace impurity by system 3. HPLC revealed a major

peak and three impurities with a combined area of 1.4% relative to the major peak area. Major peak comparisons of lot P3186-D5 with lot 127-7809 using the methods previously described but with a mobile phase of water:methanol (47:53), 3.0 mg/mL butyrophenone as the internal standard, and a flow rate of 1.2 mL/minute indicated a purity of 100.3% ± 0.6% for lot P3186-D5 relative to lot 127-7809. The overall purity of lot P3186-D5 was determined to be greater than or equal to 98%.

For lot P9189-J1, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for phenolphthalein. Karl Fischer water analysis indicated 0.12% ± 0.05% water. Functional group titration indicated a purity of 99.9% ± 0.5%. TLC indicated a major spot and a trace impurity by system 1 and only a major spot by system 3. HPLC revealed a major peak and three impurities with a combined area of 1.2% relative to the major peak area. Major peak comparisons of lot P9189-J1 with lot 127-7809 using the methods previously described but with a mobile phase of water:methanol (50:50) and 3.0 mg/mL butyrophenone as the internal standard indicated a purity of 100.9 ± 0.2% for lot P9189-J1 relative to lot 127-7809. The overall purity of lot P9189-J1 was determined to be greater than or equal to 99%.

Stability studies of lot 127-7809 were performed by the analytical chemistry laboratory. Samples were dissolved in methanol (100 mL) containing *n*-butyrophenone (1.9 mg/mL) as an internal standard. HPLC was performed as described for the purity analyses but with a mobile phase ratio of 34:63. These studies indicated that phenolphthalein was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored protected from light at 25° C in sealed containers during the 13-week studies and was stored protected from light at or below 27° C in sealed containers during the 2-year studies. Stability was monitored using HPLC approximately monthly during the 13-week studies and approximately every 16 weeks during the 2-year studies. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations for all studies were prepared weekly by mixing phenolphthalein with feed (Table J1). For the 14-day studies, dose formulations were mixed by hand for 1 minute and then blended in a Vortex feed mixer for 20 minutes using an intensifier bar for the initial 5 minutes. Formulations were stored in plastic bags within metal containers at room temperature for up to 1 week. For the 13-week and 2-year studies, a phenolphthalein/feed premix was prepared by hand and was then blended with feed in a Patterson-Kelly twin-shell blender for 15 minutes using an intensifier bar for the initial 5 minutes. For the 13-week studies, formulations were stored in double plastic bags at temperatures at or below −20° C for up to 3 weeks. For the 2-year studies, formulations were stored in polyethylene bags at room temperature protected from light for up to 3 weeks.

Stability studies of the 6,000 ppm dose formulation of lot 127-7809 were performed by the analytical chemistry laboratory. Samples were extracted with 100 mL of acetonitrile:water:hydrochloric acid (97:2:1). The samples were sonicated for 1 minute and then shaken for 20 minutes on a Burrell wrist-action shaker. After centrifugation, 5-mL aliquots were diluted to 50 mL with acetonitrile. The solutions were filtered and then analyzed using HPLC with the same system as described for the purity analyses but with a mobile phase of acetonitrile:water (45:55). The stability of the dose formulation was confirmed for at least 2 weeks when stored at temperatures up to 25° C.

Homogeneity studies of the 500 ppm dose formulation of lot P3186-D5 were performed by the analytical chemistry laboratory. Samples were extracted with 100 mL of acetonitrile:water:hydrochloric acid (97:2:1). The samples were shaken for 15 minutes on a wrist-action shaker. After centrifugation, 5-mL aliquots were mixed with 5 mL of internal standard solution (acetophenone, 0.09 mg/mL in water:

acetonitrile [45:55]) and diluted to 50 mL with water:acetonitrile (44:55). The solutions were filtered and analyzed using HPLC with a Hamilton PRP-1 10 μ column using ultraviolet detection (280 nm) and a mobile phase of water:acetonitrile (45:55). The flow rate was 1.0 mL/minute. Stability studies of the 500 ppm dose formulation were also performed using the same HPLC methods used for the homogeneity analyses. Homogeneity was confirmed and the stability of the dose formulation was confirmed for at least 3 weeks when stored protected from light at room temperature.

Periodic analyses of the dose formulations of phenolphthalein were conducted at the study laboratories using HPLC with the same methods used for the homogeneity analyses. During the 13-week studies, the formulations were analyzed every 6 weeks (Table J2). During the 2-year studies, the formulations were analyzed approximately every 8 weeks (Table J3). All of the dose formulations analyzed during the 13-week studies were within 10% of the target concentrations. Of the dose formulations analyzed during the 2-year studies, 99% (122/123) were within 10% of the target concentrations. During the 2-year studies, one 25,000 ppm sample mixed on 6 November 1992 was determined to be 28% greater than the target concentration. This formulation was remixed on 10 November 1992; the remix was within 10% of the target concentration. During the 2-year studies, 89% (40/45) of the animal room samples were within 10% of the target concentrations. One sample collected on 24 February 1992 was 52% less than the target concentration; it was determined that the food container was mislabeled and that the animals received the correct dose formulation. The other four animal room samples were within 17% of the target concentrations. Results of periodic referee analyses performed during the 13-week studies by the analytical chemistry laboratory agreed with the results obtained by the study laboratory (Table J4).

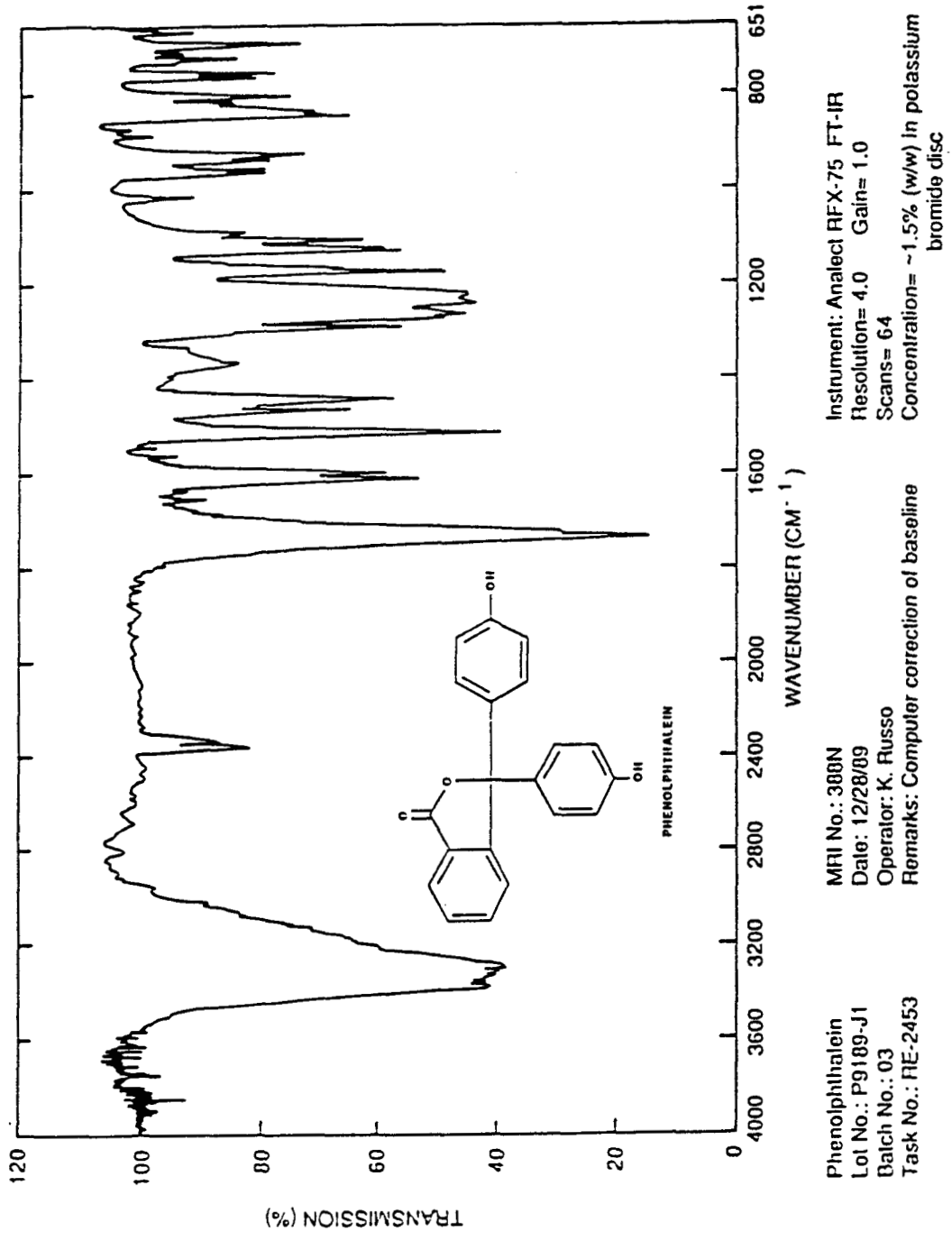

FIGURE J1
Infrared Absorption Spectrum of Phenolphthalein

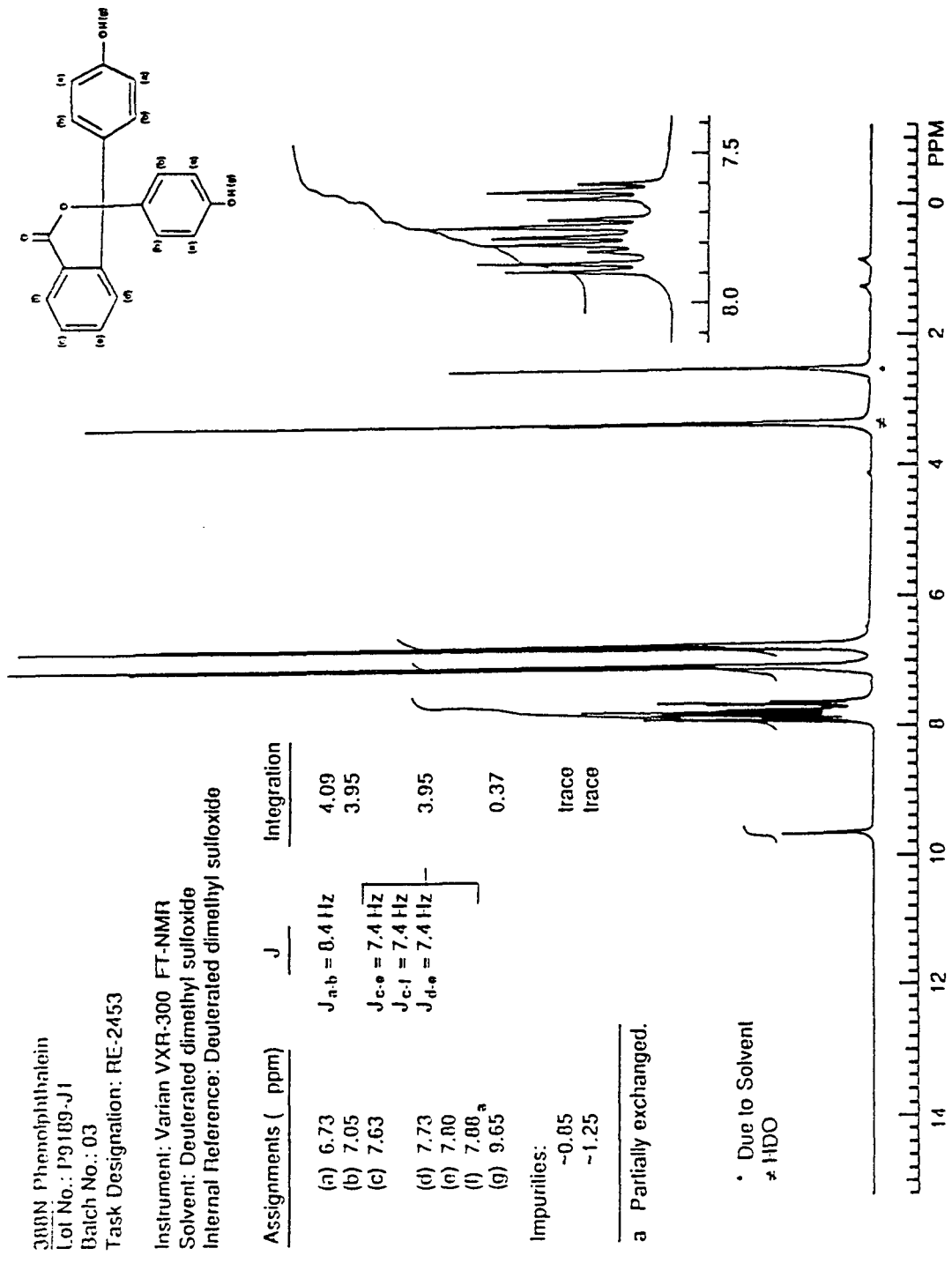

FIGURE J2
Nuclear Magnetic Resonance Spectrum of Phenolphthalein

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

14-Day Studies	13-Week Studies	2-Year Studies
Preparation		
A premix of feed and phenolphthalein was prepared by hand and then blended in a Vortex feed mixer with the intensifier bar on for 5 minutes and off for 15 minutes. Dose formulations were prepared weekly.	A premix of feed and phenolphthalein was prepared, and then layered into the remaining feed and blended in a Patterson-Kelly twin-shell blender with the intensifier bar on for 5 minutes and off for 10 minutes. Dose formulations were prepared weekly.	Same as 13-week studies
Chemical Lot Number		
127-7809	P3186-D5	P9189-J1
Maximum Storage Time		
1 week	3 weeks	3 weeks
Storage Conditions		
Stored in plastic bags within metal containers at room temperature	Stored in double plastic bags at or below −20° C	Stored in polyethylene bags protected from light at room temperature
Study Laboratory		
Cannon Laboratories, Inc. (Reading, PA)	Microbiological Associates, Inc. (Bethesda, MD)	TSI Mason Laboratories (Worcester, MA)
Referee Laboratory		
None	Midwest Research Institute (Kansas City, MO)	None

TABLE J2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 13-Week Feed Studies of Phenolphthalein

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	% Difference from Target
20 April 1987[b]	20-21 April 1987	3,000[c]	2,910	−3
		3,000[d]	2,890	−4
		3,000[e]	2,940	−2
		50,000[c]	48,800	−2
		50,000[d]	50,200	0
		50,000[e]	49,200	−2
22 April 1987	24 April 1987	3,000	3,040	+1
		6,000	6,000	0
		12,000	11,800	−2
		25,000	24,500	−2
		50,000	50,400	+1
3 June 1987	3-4 June 1987	3,000	3,000	0
		6,000	5,860	−2
		12,000	11,800	−2
		25,000	26,300	+5
		50,000	49,100	−2
15 July 1987	16-17 July 1987	3,000	2,990	0
		6,000	5,910	−2
		12,000	11,200	−7
		25,000	23,400	−6
		50,000	46,800	−6

[a] Results of duplicate analyses
[b] Homogeneity analyses, not used for dosing
[c] Sample selection from top left of twin-shell blender
[d] Sample selection from top right of twin-shell blender
[e] Sample selection from bottom of twin-shell blender

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

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	% Difference from Target
Rats				
1 November 1990[b]	2 November 1990	3,000[c]	2,980	−1
		3,000[d]	2,890	−4
		3,000[e]	2,870	−4
		50,000[c]	50,600	+1
		50,000[d]	50,400	+1
		50,000[e]	50,500	+1
1 March 1991	4 March 1991	12,000	12,300	+3
		12,000	11,700	−3
	1-2 April 1991[f]	12,000	11,200	−7
		12,000	11,600	−3
4 March 1991	4 March 1991	25,000	25,000	0
		25,000	24,700	−1
		50,000	49,800	0
		50,000	48,700	−3
	1-2 April 1991[f]	25,000	25,300	+1
		25,000	25,000	0
		50,000	50,200	0
		50,000	49,900	0
26 April 1991	29-30 April 1991	12,000	12,000	0
		12,000	12,400	+3
		25,000	25,600	+2
		25,000	25,100	0
		50,000	50,000	0
		50,000	49,900	0
21 June 1991	24-25 June 1991	12,000	12,100	+1
		12,000	12,200	+2
		25,000	25,000	0
		25,000	25,000	0
		50,000	50,400	+1
		50,000	50,000	0
16 August 1991	20 August 1991	12,000	11,900	−1
		12,000	11,800	−2
		25,000	25,500	+2
		25,000	25,900	+4
		50,000	50,500	+1
		50,000	51,500	+3
	4 September 1991[f]	12,000	11,500	−4
		12,000	12,000	0
		25,000	25,400	+2
		25,000	25,000	0
		50,000	49,500	−1
		50,000	49,600	−1

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Phenolphthalein (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
Rats (continued)				
11 October 1991	15 October 1991	12,000	11,500	−4
		25,000	24,500	−2
		50,000	50,000	0
5 December 1991	6 December 1991	12,000	11,700	−3
		12,000	11,700	−3
		25,000	25,100	0
		25,000	24,800	−1
		50,000	49,000	−2
		50,000	48,300	−3
31 January 1992	3 February 1992	12,000	12,300	+3
		25,000	25,400	+2
		50,000	49,700	−1
	24 February 1992[f]	12,000	12,100	+1
		25,000	11,900	−52[g]
		50,000	50,000	0
27 March 1992	30 March 1992	12,000	12,200	+2
		12,000	12,300	+3
		25,000	25,500	+2
		25,000	25,700	+3
		50,000	49,700	−1
		50,000	51,200	+2
22 May 1992	26 May 1992	12,000	12,100	+1
		12,000	12,200	+2
		25,000	24,600	−2
		25,000	24,200	−3
		50,000	47,000	−6
		50,000	47,500	−5
17 July 1992	20 July 1992	12,000	11,700	−3
		12,000	11,600	−3
		25,000	23,900	−4
		25,000	24,200	−3
		50,000	49,600	−1
		50,000	53,300	+7
	10-12 August 1992[f]	12,000	12,000	0
		12,000	11,800	−2
		25,000	24,900	0
		25,000	24,500	−2
		50,000	49,500	−1
		50,000	49,000	−2

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Phenolphthalein (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
Rats (continued)				
18 September 1992	21 September 1992	12,000	12,000	0
		12,000	12,000	0
		25,000	24,900	0
		25,000	25,000	0
		50,000	50,100	0
		50,000	50,300	+1
6 November 1992	9 November 1992	12,000	12,100	+1
		12,000	12,000	0
		25,000	25,200	+1
		25,000	31,900	+28
		50,000	49,800	0
		50,000	50,300	+1
10 November 1992[h]	10 November 1992	25,000	26,200	+5
4 January 1993	5 January 1993	12,000	10,900	−9
		12,000	10,800	−10
		25,000	25,800	+3
		25,000	25,100	0
		50,000	49,200	−2
		50,000	49,400	−1
	25 January 1993[f]	12,000	11,400	−5
		12,000	11,300	−6
		25,000	25,100	0
		25,000	24,700	−1
		50,000	49,000	−2
		50,000	50,000	0
19 February 1993	22 February 1993	12,000	12,000	0
		12,000	12,400	+3
		25,000	25,800	+3
		25,000	25,400	+2
		50,000	48,300	−3
		50,000	48,500	−3
Mice				
1 November 1990[b]	2 November 1990	3,000[c]	2,980	−1
		3,000[d]	2,890	−4
		3,000[e]	2,870	−4
		50,000[c]	50,600	+1
		50,000[d]	50,400	+1
		50,000[e]	50,500	+1

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Phenolphthalein (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
Mice (continued)				
16 November 1990	19 November 1990	3,000	2,970	−1
		3,000	3,060	+2
		6,000	6,020	0
		6,000	6,020	0
		12,000	12,040	0
		12,000	12,150	+1
	11 December 1990[f]	3,000	2,500	−17[i]
		3,000	2,750	−8[i]
		6,000	5,370	−11[i]
		6,000	5,470	−9[i]
		12,000	10,080	−16[i]
		12,000	11,350	−5[i]
18 January 1991	21 January 1991	3,000	3,060	+2
		6,000	6,030	+1
		12,000	12,040	0
8 March 1991	11 March 1991	3,000	3,000	0
		6,000	6,100	+2
		12,000	11,990	0
26 April 1991	29 April 1991	3,000	3,090	+3
		6,000	5,970	−1
		12,000	11,870	−1
	16 May 1991[f]	3,000	2,640	−12[i]
		6,000	5,520	−8
		12,000	11,670	−3
21 June 1991	24 June 1991	3,000	3,050	+2
		6,000	5,930	−1
		12,000	11,960	0
16 August 1991	20 August 1991	3,000	3,000	0
		6,000	6,050	+1
		12,000	12,160	+1
11 October 1991	15 October 1991	3,000	2,940	−2
		6,000	5,980	0
		12,000	11,950	0
	30 October 1991[f]	3,000	2,920	−3
		6,000	5,800	−3
		12,000	11,900	−1
5 December 1991	6 December 1991	3,000	3,010	0
		6,000	5,990	0
		12,000	11,900	−1

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Phenolphthalein (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
Mice (continued)				
31 January 1992	3 February 1992	3,000	3,090	+3
		6,000	6,100	+2
		12,000	12,060	+1
27 March 1992	30 March 1992	3,000	3,210	+7
		6,000	5,900	−2
		12,000	11,880	−1
	15-16 April 1992[f]	3,000	3,150	+5
		6,000	6,240	+4
		12,000	12,170	+1
22 May 1992	22 May 1992	3,000	3,060	+2
		6,000	6,030	+1
		12,000	11,960	0
17 July 1992	20 July 1992	3,000	2,920	−3
		6,000	6,050	+1
		12,000	11,890	−1
18 September 1992	21 September 1992	3,000	3,080	+3
		6,000	6,050	+1
		12,000	12,020	0
	10 October 1992[f]	3,000	2,910	−3
		6,000	5,800	−3
		12,000	12,030	0
6 November 1992	9 November 1992	3,000	3,040	+1
		6,000	6,130	+2
		12,000	12,080	+1

[a] Results of duplicate analyses
[b] Homogeneity analyses, not used for dosing
[c] Sample selection from top left of twin-shell blender
[d] Sample selection from top right of twin-shell blender
[e] Sample selection from bottom of twin-shell blender
[f] Animal room samples
[g] For the 25,000 ppm dose formulation prepared 31 January 1992, it was determined that a container of the 12,000 ppm dose formulation was mislabeled when the animal room samples were collected on 24 February 1992 and that the animals were not misdosed.
[h] Results of remix
[i] Low values were caused by the presence of feces, urine, and/or bedding in the animal room samples; it was determined that the animals were not misdosed.

TABLE J4
Results of Referee Analyses of Dose Formulations Administered to Rats and Mice in the 13-Week Feed Studies of Phenolphthalein

Date Prepared	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory[a]	Referee Laboratory[b]
22 April 1987	6,000	6,000	6,100 ± 60
15 July 1987	25,000	23,400	25,500 ± 300

[a] Results of duplicate analyses
[b] Results of triplicate analyses (mean ± standard error)

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

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

Week	0 ppm		12,000 ppm			25,000 ppm			50,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
2	14.5	170	12.7	168	907	12.7	168	1,899	12.3	161	3,818
4	17.3	237	17.6	229	924	19.6	233	2,105	18.4	227	4,051
5	16.8	258	17.5	250	840	18.2	254	1,790	18.0	249	3,604
9	17.4	320	16.3	301	651	16.5	294	1,403	18.0	306	2,949
13	15.5	344	17.0	327	622	17.9	326	1,372	16.3	333	2,453
17	16.8	382	17.0	364	562	17.3	362	1,198	17.6	365	2,416
25	16.0	415	17.3	398	520	17.8	402	1,106	18.3	405	2,256
29	15.7	425	16.7	406	492	17.4	410	1,059	16.9	409	2,068
33	15.8	437	18.4	411	537	17.9	421	1,062	18.0	420	2,136
37	16.2	449	17.4	428	487	18.1	432	1,048	17.9	434	2,058
41	16.8	459	18.3	437	503	18.4	442	1,039	18.6	443	2,100
45	15.9	460	17.5	440	476	17.7	442	1,002	18.0	443	2,029
49	16.2	463				19.0	446	1,062	18.6	446	2,092
53	15.5	464	16.7	443	451	17.6	445	988	17.9	447	1,999
57	16.4	459	17.2	439	469	17.4	441	984	17.3	442	1,961
61	16.1	466	16.9	439	463	18.1	444	1,018	17.7	439	2,023
65	15.8	470	17.5	443	475	19.8	443	1,116	18.2	447	2,034
69	14.1	466	16.0	440	437	16.6	445	936	16.9	442	1,906
73	13.8	461	15.9	442	433	15.8	441	894	16.1	442	1,825
77	14.7	465	15.3	436	422	15.9	439	907	16.1	437	1,843
81	13.9	457	14.1	433	392	15.9	431	920	16.0	431	1,854
85	14.1	455	14.4	423	409	15.6	422	923	15.6	428	1,821
89	12.8	450	13.8	419	394	13.5	404	834	14.3	415	1,719
93	14.1	457	14.4	423	407	14.0	397	884	13.7	390	1,758
97	13.5	451	14.6	405	432	15.2	395	961	14.5	409	1,780
101	14.0	439	14.0	375	448	14.3	364	979	13.5	372	1,822
104	12.8	434	13.7	367	448	13.6	341	998	13.5	342	1,972
Mean for weeks											
1-13	16.3	266	16.2	255	789	17.0	255	1,714	16.6	255	3,375
14-52	16.2	436	17.5	412	511	17.9	420	1,072	18.0	421	2,144
53-104	14.4	457	15.3	423	434	15.9	418	953	15.8	420	1,880

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

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

Week	0 ppm		12,000 ppm			25,000 ppm			50,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
2	10.4	129	9.3	129	865	9.4	128	1,829	9.4	129	3,661
4	9.7	142	11.1	147	907	11.1	146	1,896	11.6	151	3,844
5	11.9	157	11.0	157	843	10.9	155	1,764	11.0	159	3,441
9	11.0	184	10.6	182	702	10.5	179	1,461	11.3	180	3,144
13	10.5	201	10.1	195	618	10.4	196	1,324	11.5	191	3,024
16	10.7	214	11.0	204	647	10.6	202	1,313	11.1	200	2,775
21	10.9	222	10.7	211	609	10.6	209	1,275	11.2	206	2,712
25	10.7	229	11.3	220	615	11.1	216	1,289	11.4	214	2,677
29	10.8	234	10.8	222	583	10.6	219	1,215	10.8	216	2,499
33	10.1	240	11.1	225	593	11.0	223	1,235	11.2	219	2,566
37	11.7	252	11.4	234	584	11.2	230	1,217	11.6	228	2,548
41	11.8	261	12.2	240	613	11.8	238	1,241	12.3	234	2,624
45	11.4	267	11.3	244	556	11.6	242	1,196	11.8	237	2,480
49	11.4	281	12.1	254	572	11.8	249	1,189	12.2	245	2,497
53	11.5	287	11.6	258	543	11.6	254	1,147	11.7	249	2,355
57	11.8	296	11.6	262	530	11.5	256	1,122	12.0	251	2,396
61	12.0	304	11.5	265	520	11.6	260	1,119	12.0	259	2,323
65	11.9	314	11.9	272	522	12.5	269	1,160	12.3	265	2,320
69	11.4	322	11.4	282	485	11.7	280	1,047	11.5	270	2,128
73	11.9	325	11.7	287	490	11.9	284	1,050	12.1	275	2,198
77	12.2	332	11.9	290	491	12.1	288	1,048	12.3	281	2,193
81	12.8	342	12.5	298	503	12.2	293	1,039	11.9	284	2,090
85	11.2	346	11.0	303	438	11.4	296	961	12.0	292	2,059
89	11.3	351	10.9	301	436	10.7	295	902	11.2	291	1,918
93	10.7	345	10.4	299	417	10.8	294	914	11.5	293	1,967
97	9.7	346	9.9	290	410	10.6	298	893	11.3	290	1,940
101	10.4	348	11.1	301	441	10.5	300	877	11.5	296	1,941
105	10.5	337	11.4	302	455	10.9	303	904	11.3	294	1,923
Mean for weeks											
1-13	10.7	162	10.4	162	787	10.4	161	1,655	11.0	162	3,423
14-52	11.1	244	11.3	228	597	11.2	225	1,241	11.5	222	2,598
53-105	11.4	328	11.3	286	477	11.4	284	1,013	11.8	278	2,125

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

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

Week	0 ppm		3,000 ppm			6,000 ppm			12,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
2	4.2	25.2	4.4	24.9	530	4.1	24.8	1,002	4.1	24.8	1,999
5	4.2	27.7	4.1	27.4	447	4.2	27.1	933	4.2	26.9	1,855
9	4.6	29.5	4.3	29.2	441	5.0	29.4	1,025	4.3	28.8	1,809
13	4.1	32.6	4.3	32.8	393	4.4	32.6	801	4.2	32.5	1,547
17	4.2	35.2	4.3	35.0	367	4.4	34.7	763	3.8	34.2	1,328
21	4.3	40.5	4.4	39.1	336	4.3	39.7	644	4.2	38.5	1,305
25	4.0	42.8	4.1	41.6	295	4.3	41.8	611	4.2	41.2	1,228
29	4.2	44.8	4.7	43.9	320	4.6	44.0	630	4.5	43.2	1,256
33	4.2	46.5	4.4	45.7	287	4.6	45.9	598	4.6	43.8	1,248
37	4.6	46.8	4.5	46.0	293	4.6	45.7	598	4.3	43.9	1,188
41	4.7	46.2	4.7	45.0	313	4.5	45.3	591	4.6	44.9	1,231
45	4.4	47.1	4.6	47.0	291	4.8	46.4	616	4.8	45.6	1,259
49	4.7	48.7	4.8	48.2	301	4.8	47.7	610	4.7	46.9	1,206
53	4.6	48.7	4.7	48.3	289	4.9	48.3	608	4.7	47.0	1,196
57	4.7	49.8	4.7	49.0	288	4.9	48.9	600	4.7	47.5	1,194
61	5.2	49.6	4.9	48.9	302	5.3	47.9	659	5.1	47.3	1,302
65	4.9	51.7	5.0	50.5	298	5.2	49.8	621	4.9	49.8	1,191
69	4.8	52.0	4.9	50.9	289	5.0	49.8	601	4.8	49.8	1,149
73	5.1	51.7	5.1	50.7	303	5.2	51.0	607	4.9	50.3	1,179
77	4.7	51.6	4.8	50.7	282	4.7	50.3	565	4.8	49.8	1,157
81	4.7	51.1	4.6	50.2	277	4.8	51.0	567	4.7	49.8	1,130
85	4.6	51.1	4.8	50.2	287	4.9	51.5	575	4.8	50.1	1,153
89	4.3	51.5	4.7	49.5	282	4.9	50.8	573	4.8	49.4	1,162
93	5.3	50.0	5.2	49.1	320	5.2	50.2	622	5.0	47.1	1,261
97	4.9	50.4	4.9	49.1	298	4.8	50.1	575	4.9	47.3	1,238
101	4.7	49.9	4.6	49.0	284	4.6	50.2	549	4.5	46.3	1,157
104	4.6	49.4	4.4	47.9	279	4.3	49.1	525	4.1	45.9	1,062
Mean for weeks											
1-13	4.3	28.8	4.3	28.6	453	4.4	28.5	940	4.2	28.3	1,802
14-52	4.4	44.3	4.5	43.5	311	4.5	43.5	629	4.4	42.5	1,250
53-104	4.8	50.6	4.8	49.6	291	4.9	49.9	589	4.8	48.4	1,181

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

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

Week	0 ppm		3,000 ppm			6,000 ppm			12,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
2	6.2	19.9	5.3	19.5	812	6.0	19.6	1,831	5.4	19.6	3,299
5	4.7	21.8	4.8	21.8	658	4.7	21.7	1,301	4.4	21.9	2,409
9	5.2	24.9	5.2	24.3	645	5.6	24.7	1,351	5.7	24.9	2,733
13	4.2	27.0	4.3	26.1	489	5.1	26.9	1,144	4.8	27.4	2,118
17	4.3	30.6	4.5	29.1	460	4.8	29.3	982	4.5	29.6	1,844
21	4.7	34.0	4.9	33.6	436	4.7	33.6	843	4.8	34.3	1,666
25	4.3	36.2	4.6	35.2	392	4.4	35.1	757	4.2	35.8	1,409
29	4.7	38.8	4.7	37.5	373	4.7	37.8	750	4.6	37.5	1,475
33	4.7	42.2	4.8	40.3	359	4.7	40.3	699	4.8	39.9	1,433
37	4.7	42.1	4.7	40.6	346	4.8	40.7	701	4.7	40.2	1,407
41	5.0	44.5	4.9	42.8	344	4.9	42.1	696	4.9	41.7	1,397
45	5.1	45.2	5.3	43.1	366	5.1	42.9	719	5.2	43.1	1,444
49	4.9	46.6	5.2	44.6	349	5.1	43.7	702	5.0	43.0	1,406
53	5.0	47.8	4.7	46.1	308	4.9	45.5	643	4.9	45.0	1,311
57	5.1	49.0	5.2	46.9	334	5.1	47.1	654	5.1	46.5	1,326
61	5.7	50.2	5.7	48.0	354	5.5	47.6	697	5.4	46.8	1,384
65	5.5	53.6	5.6	50.7	332	5.6	51.1	656	5.3	49.7	1,270
69	5.4	55.6	5.5	52.3	317	5.2	51.0	615	5.4	50.8	1,271
73	5.7	55.6	5.7	52.9	325	5.7	51.7	659	5.3	50.9	1,245
77	5.0	54.6	5.1	52.4	294	4.9	51.4	567	5.2	50.3	1,244
81	5.1	55.6	4.9	52.7	282	5.0	51.5	582	5.1	50.3	1,210
85	5.2	56.3	5.2	52.9	295	5.0	52.5	566	5.0	51.2	1,169
89	5.3	56.5	5.4	52.6	309	5.3	52.7	608	5.6	52.9	1,260
93	5.8	55.2	5.6	51.4	330	5.6	51.0	659	5.5	51.7	1,287
97	5.5	55.6	5.4	52.5	307	5.3	51.8	615	5.4	51.8	1,246
101	5.1	54.9	4.8	50.3	288	4.8	52.0	550	5.1	51.6	1,190
105	4.9	54.0	4.7	49.4	283	4.6	49.3	564	4.9	50.5	1,155
Mean for weeks											
1-13	5.1	23.4	4.9	22.9	651	5.3	23.2	1,407	5.1	23.5	2,640
14-52	4.7	40.0	4.8	38.5	381	4.8	38.4	761	4.7	38.3	1,498
53-105	5.3	53.9	5.3	50.8	311	5.2	50.4	617	5.2	50.0	1,255

[a] Grams of feed consumed per animal per day
[b] Milligrams of phenolphthalein 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[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 were 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)	23.61 ± 0.52	22.6 — 25.2	25
Crude fat (% by weight)	5.28 ± 0.24	4.80 — 5.80	25
Crude fiber (% by weight)	3.34 ± 0.29	2.60 — 3.80	25
Ash (% by weight)	6.54 ± 0.21	6.23 — 7.03	25
Amino Acids (% of total diet)			
Arginine	1.280 ± 0.083	1.110 — 1.390	11
Cystine	0.308 ± 0.071	0.181 — 0.400	11
Glycine	1.158 ± 0.048	1.060 — 1.220	11
Histidine	0.584 ± 0.027	0.531 — 0.630	11
Isoleucine	0.917 ± 0.033	0.867 — 0.965	11
Leucine	1.975 ± 0.051	1.850 — 2.040	11
Lysine	1.274 ± 0.049	1.200 — 1.370	11
Methionine	0.437 ± 0.109	0.306 — 0.699	11
Phenylalanine	0.999 ± 0.120	0.665 — 1.110	11
Threonine	0.904 ± 0.058	0.824 — 0.985	11
Tryptophan	0.218 ± 0.153	0.107 — 0.671	11
Tyrosine	0.685 ± 0.094	0.564 — 0.794	11
Valine	1.086 ± 0.055	0.962 — 1.170	11
Essential Fatty Acids (% of total diet)			
Linoleic	2.407 ± 0.227	1.830 — 2.570	10
Linolenic	0.259 ± 0.065	0.100 — 0.320	10
Vitamins			
Vitamin A (IU/kg)	6,976 ± 1,367	5,940 — 12,540	25
Vitamin D (IU/kg)	4,450 ± 1,382	3,000 — 6,300	4
α-Tocopherol (ppm)	36.12 ± 9.15	22.5 — 48.9	10
Thiamine (ppm)	17.76 ± 2.76	12.0 — 25.0	25
Riboflavin (ppm)	7.83 ± 0.923	6.10 — 9.00	11
Niacin (ppm)	98.64 ± 25.5	65.0 — 150.0	10
Pantothenic acid (ppm)	30.55 ± 3.52	23.0 — 34.6	11
Pyridoxine (ppm)	9.11 ± 2.53	5.60 — 14.0	11
Folic acid (ppm)	2.46 ± 0.63	1.80 — 3.70	11
Biotin (ppm)	0.268 ± 0.047	0.190 — 0.354	11
Vitamin B_{12} (ppb)	40.5 ± 19.1	10.6 — 65.0	11
Choline (ppm)	2,991 ± 382	2,300 — 3,430	10
Minerals			
Calcium (%)	1.18 ± 0.09	1.02 — 1.36	25
Phosphorus (%)	0.93 ± 0.060	0.770 — 1.03	25
Potassium (%)	0.886 ± 0.063	0.772 — 0.971	9
Chloride (%)	0.529 ± 0.087	0.380 — 0.635	9
Sodium (%)	0.316 ± 0.033	0.258 — 0.371	11
Magnesium (%)	0.166 ± 0.010	0.148 — 0.181	11
Sulfur (%)	0.272 ± 0.059	0.208 — 0.420	10
Iron (ppm)	350.5 ± 87.3	255.0 — 523.0	11
Manganese (ppm)	92.48 ± 5.14	81.7 — 99.4	11
Zinc (ppm)	59.33 ± 10.2	46.1 — 81.6	11
Copper (ppm)	11.81 ± 2.50	8.09 — 15.4	11
Iodine (ppm)	3.54 ± 1.19	1.52 — 5.83	10
Chromium (ppm)	1.66 ± 0.46	0.85 — 2.09	11
Cobalt (ppm)	0.76 ± 0.23	0.49 — 1.15	7

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 ± 0.20	0.10 — 0.80	25
Cadmium (ppm)	0.14 ± 0.08	0.04 — 0.20	25
Lead (ppm)	0.31 ± 0.22	0.10 — 1.10	25
Mercury (ppm)	0.02 ± 0.00	0.02 — 0.03	25
Selenium (ppm)	0.35 ± 0.08	0.10 — 0.40	25
Aflatoxins (ppb)	<5.0		25
Nitrate nitrogen (ppm)[c]	7.20 ± 3.29	1.80 — 16.0	25
Nitrite nitrogen (ppm)[c]	0.17 ± 0.12	0.02 — 0.50	25
BHA (ppm)[d]	1.40 ± 0.91	1.00 — 4.00	25
BHT (ppm)[d]	1.36 ± 1.22	1.00 — 7.00	25
Aerobic plate count (CFU/g)	135,520 ± 167,289	10,000 — 630,000	25
Coliform (MPN/g)	75 ± 232	3 — 1,100	25
Escherichia coli (MPN/g)	<3		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	7.03 ± 1.75	4.80 — 11.10	25
N-Nitrosodimethylamine (ppb)[e]	5.06 ± 1.12	3.40 — 7.90	25
N-Nitrosopyrrolidine (ppb)[e]	1.99 ± 1.20	1.00 — 5.80	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.20 ± 0.18	0.05 — 0.54	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

[a] CFU=colony forming units, MPN=most probable number, BHC is 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

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 13-week and 2-year studies of phenolphthalein. 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	
13-Week Study	
ELISA	
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/ sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	Study termination
KRV (Kilham rat virus)	Study termination
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM	5, 9, 12, 17, 18, and 23 months, study termination
RCV/SDA	5, 9, 12, 17, 18, and 23 months, study termination
Sendai	5, 9, 12, 17, 18, and 23 months, study termination
Immunofluorescence Assay	
RCV/SDA	Study termination
Hemagglutination Inhibition	
H-1	5, 9, 12, 17, 18, and 23 months, study termination
KRV	5, 9, 12, 17, 18, and 23 months, study termination

MICE

13-Week Study

ELISA	
Ectromelia virus	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
MVM (minute virus of mice)	Study termination
Mouse adenoma virus	Study termination
MHV (mouse hepatitis virus)	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Immunofluorescence Assay	
EDIM (epizootic diarrhea of infant mice)	Study termination
Hemagglutination Inhibition	
K (papovavirus)	Study termination
Polyoma virus	Study termination

2-Year Study

ELISA	
Ectromelia virus	5, 12, and 18 months, study termination
EDIM	12 and 18 months, study termination
GDVII	5, 12, and 18 months, study termination
LCM	5, 12, and 18 months, study termination
Mouse adenoma virus-FL	5, 12, and 18 months, study termination
MHV	5, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	5, 12, and 18 months, study termination
Reovirus 3	5, 12, and 18 months, study termination
Sendai	5, 12, and 18 months, study termination
Immunofluorescence Assay	
EDIM	5 months, study termination
GDVII	Study termination
MHV	12 months
Reovirus 3	12 months
Hemagglutination Inhibition	
K	5, 12, and 18 months, study termination
MVM	5, 12, and 18 months, study termination
Polyoma virus	5, 12, and 18 months, study termination

RESULTS

One rat had a positive titer to *M. arthritidis* at the end of the 2-year study. Further evaluation of the serum positive for *M. arthritidis* by immunoblot and Western blot procedures indicated that the positive titer may have been due to a cross reaction with antibodies of nonpathogenic *Mycoplasma* or other agents. There were no clinical findings or histopathologic changes indicative of *M. arthritidis* infection in the rat with the positive titer. Accordingly, the *M. arthritidis*-positive titer was considered to be a false positive.

APPENDIX N
CONTINUOUS BREEDING STUDY IN SWISS (CD-1®) MICE

CONTINUOUS BREEDING STUDY IN SWISS (CD-1®) MICE

INTRODUCTION

The potential reproductive toxicity of phenolphthalein was evaluated in Swiss (CD-1®) mice because evidence in the literature suggests that phenolphthalein has a weak estrogenic activity (Bitman and Cecil, 1970; Ravdin *et al.*, 1987) and because data gathered at the end of the 13-week study in B6C3F_1 mice indicate chemical-related effects in male mice (Appendix I). The effects of exposure to phenolphthalein on reproduction were assessed with a continuous breeding study in Swiss (CD-1®) mice administered phenolphthalein in feed (NTP, 1991).

Reproductive assessment by the methods presented in Lamb (1985) and Heindel *et al.* (1989) consists of four phases: dose range finding, continuous breeding, identification of the affected sex (crossover mating trial), and offspring assessment. A 2-week dose-finding phase is conducted to determine exposure concentrations of the continuous breeding phase. During the continuous breeding phase, the effects of the maximum tolerated exposure concentration estimated in the dose-finding phase and two lower exposure concentrations on fertility and reproduction of first-generation (F_0) animals are determined. If fertility is significantly affected during the continuous breeding phase, crossover mating trials are performed to determine if males, females, or both sexes are affected. Offspring assessment includes evaluation of reproductive performance of second-generation (F_1) animals from the final litters of the continuous breeding phase. The F_1 animals are raised to sexual maturity while receiving the same exposure concentrations as their parents, are mated, and are allowed to deliver the third-generation (F_2) offspring.

In the phenolphthalein study, the exposure concentrations for the continuous breeding study were based on results of studies reported in the literature. A crossover mating trial was performed, and pups from all exposure groups were maintained for offspring assessment.

MATERIALS AND METHODS

Phenolphthalein was obtained from Pharmco Laboratories, Inc. (Titusville, FL) in one lot (P3186-D5), which was also used for the 13-week studies conducted at Microbiological Associates, Inc. (Bethesda, MD). Results of purity and stability analyses of lot P3186-D5 are given in Appendix J. Dose formulations were prepared every 3 weeks, and analyses by Research Triangle Institute indicated that the dose formulations were homogeneous and within 10% of the target concentration.

Male and female VAF Crl:Swiss CD-1® (ICR)BR outbred albino mice were obtained from Charles River Breeding Laboratories, Inc. (Kingston, NY). Upon receipt, serum samples were collected from two males and two females, and the serum samples were analyzed for antibody titers to rodent titers. All serum samples were negative. Mice were quarantined for 2 weeks and were 11 weeks old at the start of the continuous breeding study. Mice were housed two per cage by sex during quarantine. NIH-07 open formula meal diet containing the appropriate concentrations of phenolphthalein and distilled water were available *ad libitum* for all phases of the study.

During the continuous breeding phase, the mice were housed two per cage by sex for 1 week while being exposed to 0, 1,000, 7,000, or 30,000 ppm phenolphthalein; mice were then housed in breeding pairs for 98 days while exposure continued. After the mating period, mice were housed separately for approximately 21 days while exposure continued to allow delivery of the final litter of pups. During the continuous breeding phase, clinical observations, feed consumption, pregnancy index, litters per pair, length of gestation, dam body weights, live pups per litter, proportion of pups born alive, sex of live

pups, and pup body weights were recorded (Tables N1 and N2). For the last litter, pup survival and body weights were recorded on lactation days 0, 4, 7, 14, and 21 (Table N3).

After the last litter of pups born during the holding period was weaned, crossover mating trials with the F_0 adult mice were performed. The following groups were mated: 0 ppm males × 0 ppm females (18 pairs), 0 ppm males × 7,000 ppm females (19 pairs), and 7,000 ppm males × 0 ppm females (18 pairs).. The breeding pairs were housed together for 7 days or until a vaginal plug was observed, after which mice were housed separately while exposure continued. Clinical observations, feed consumption, mating index, pregnancy index, fertility index, dam body weights, length of gestation, live pups per litter, proportion of pups born alive, sex of live pups, and pup body weights were recorded (Table N4). Before necropsy, estrous cycle data were collected (Table N6). At necropsy, sperm data were collected (Table N6), and the following organs were weighed: right cauda epididymis, right epididymis, kidneys (with adrenal glands), liver, right ovary, prostate gland, seminal vesicles, and right testis (Table N5). Selected organs were fixed in 10% neutral buffered formalin or Bouin's fixative and imbedded in glycol methacrylate or paraffin. Sections were stained with hematoxylin and eosin (testis only) or PAS and hematoxylin. Histopathologic examination of selected organs was performed on 10 representative 0 and 7,000 ppm males and females as well as on animals that died during the crossover mating trial (Table N7).

To assess the offspring of treated mice, the final litter of pups born to each F_0 mouse in the 0, 1,000, and 7,000 ppm groups was raised to sexual maturity. After weaning, siblings were housed two per cage by sex and were administered the same exposure concentrations as their parents. At sexual maturity (74 ± 10 days of age), nonsibling male and female mice from within the same exposure group (20 pairs per group) were housed as breeding pairs for 7 days. Female mice were examined for a copulatory plug, and mice were then housed separately through the delivery of pups. Clinical observations, feed consumption, mating index, pregnancy index, fertility index, dam body weights, length of gestation, live pups per litter, proportion of pups born alive, sex of live pups, and pup body weights were recorded (Table N8). Before necropsy, estrous cycle data were collected (Table N10). At necropsy, sperm data were collected (Table N10), and the following organs were weighed: right cauda epididymis, right epididymis, kidneys (with adrenal glands), liver, right ovary, prostate gland, seminal vesicles, and right testis (Table N9). Selected organs were fixed in 10% neutral buffered formalin or Bouin's fixative and imbedded in glycol methacrylate or paraffin. Sections were stained with hematoxylin and eosin (testis only) or PAS and hematoxylin. Histopathologic examination of selected organs was performed on 10 representative 0 and 7,000 ppm males and females (Table N11).

For data expressed as proportions (fertility, mating, and pregnancy indices), the Cochran-Armitage test (Armitage, 1971) was used to test for dose-related trends, and pairwise comparisons were performed with a chi-square test (Conover, 1971). A chi-square test for homogeneity was used to identify overall differences in fertility across exposure groups and for pairwise comparisons in the crossover mating trial.

The number of litters and the number of live pups per litter were determined per fertile pair and then exposure group means were determined. The proportion of live pups was defined as the number of pups born alive divided by the total number of pups produced by each pair. The sex ratio was expressed as the number of male pups born alive divided by the total number of live pups born to each fertile pair.

Exposure group means for data with skewed distributions were analyzed by the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Shirley's) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunn's). In the crossover mating trial, the Kruskal-Wallis test (Kruskal and Wallis, 1952) was used

to test for overall differences among exposure groups, and multiple comparisons were made with Dunn's test or Wilcoxon's test (Conover, 1971).

Analyses of covariance (Neter and Wasserman, 1974) with average litter size as the covariate, were performed to remove the potential effect of number of pups per litter on average pup weight. Least-squares estimates of exposure group means adjusted for litter size were tested for overall equality by an F-test and for pairwise equality by Dunnett's test (Dunnett, 1955) or a *t*-test; these tests were performed on males, females, and males and females (combined) to analyze potential sex differences.

For vaginal cytology data, an arcsine transformation was used to bring the data into closer conformance with normality assumptions. 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 (Tables N6 and N10).

Incidences of lesions were analyzed by the Fisher exact test.

RESULTS

During the continuous breeding phase, exposure to phenolphthalein caused significant reproductive toxicity in F_0 mice. The pregnancy indices for the 7,000 and 30,000 ppm exposure groups were significantly lower than those of the controls for most litters, and only one pair in the 30,000 ppm exposure group delivered a fifth litter (Table N1). The average numbers of litters per pair of the 7,000 and 30,000 ppm exposure groups were significantly lower than that of the controls. The cumulative days to litter for the 7,000 and 30,000 ppm exposure groups were significantly greater than that for the controls from litter 2 through litter 4. Although the body weights of exposed dams were generally similar to those of the controls at delivery (data not shown), the body weight of dams in the 30,000 ppm exposure group was significantly lower than that of the controls on lactation day 0. Body weights of dams in the 7,000 and 30,000 ppm exposure groups during lactation of litter 5 were significantly lower than those of the controls from lactation days 4 to 14, and the body weight of dams in the 7,000 ppm exposure group was significantly lower than that of the controls on lactation day 21. The numbers of live male, live female, and total live pups per litter in the 7,000 and 30,000 ppm exposure groups were significantly lower than those in the controls (Table N1). The adjusted total weight of live pups in the 30,000 ppm exposure group was significantly lower than that in the controls.

The numbers of live pups per litter in the 7,000 and 30,000 ppm exposure groups were significantly lower than those in the controls for most litters and for the combined litters (Table N2). The adjusted total weight of live pups in litters 2, 3, and 4 and in the combined litters 1 through 5 in the 30,000 ppm exposure group were significantly lower than those of the controls.

The survival of male pups in litter 5 in the 30,000 ppm exposure group was significantly lower than that in the controls on lactation days 4 through 21 (Table N3). The total survival of male and female pups in the 30,000 ppm exposure group was significantly lower than that of the controls on lactation days 7 and 14. Body weights of male pups in the 7,000 ppm exposure group were significantly lower than those of the controls on lactation days 14 and 21; the single surviving pup in the 30,000 ppm exposure group also weighed less than the control pups.

For dams in the 7,000 ppm exposure group of the crossover mating trial, the average body weight at delivery and the number of days to litter were significantly lower than those of the controls (Table N4). The numbers of live male, live female, and total live pups per litter by dams in the 7,000 ppm exposure group were significantly lower than those of the controls. There were no significant differences in

fertility, reproductive performance, or length of gestation between 0 ppm dams mated with 7,000 ppm males and those mated with 0 ppm males. Functionally, males were affected substantially less than females by exposure to phenolphthalein.

All mice in the control and 7,000 ppm F_0 groups were necropsied. In females, there were no treatment-related differences in body or organ weights (Table N5). There was no significant difference in the length or pattern of the estrous cycle between females in the control and 7,000 ppm groups (Table N6). The absolute and relative right epididymis and right testis weights of 7,000 ppm F_0 males were significantly lower than those of the controls (Table N5). The absolute weight of the kidneys (with adrenal glands) of the 7,000 ppm F_0 males was significantly greater than that of the controls. The sperm concentration in 7,000 ppm F_0 males was significantly lower than that in the controls, and the percent of abnormal sperm in this group was significantly greater than that in the controls (Table N6). There was evidence of seminiferous tubule degeneration (9/10), interstitial hyperplasia (4/10), and epididymal degeneration (7/10) in males in the 7,000 ppm exposure group (Table N7).

Because of excessive toxicity at 30,000 ppm, the reproductive effects on the F_1 mice in the offspring assessment phase were evaluated only at 0, 1,000, and 7,000 ppm. At the time of mating, there were no treatment-related differences in body weights (data not shown). The pregnancy index of the 7,000 ppm exposure group was significantly lower than that of the controls (Table N8). The length of gestation for the 7,000 ppm exposure group was significantly greater than that of the controls. In 7,000 ppm group, fewer than half of the pairs that mated delivered any live young. The numbers of live male pups and total live pups per litter and the sex ratio in the 7,000 ppm exposure group were significantly lower than those of the controls. The body weights of male, female, and total pups in the 7,000 ppm exposure group were significantly lower than those of the controls.

The necropsy body weight and absolute liver weight of 7,000 ppm F_1 females were significantly lower than those of the controls (Table N9). There were no significant differences in estrous cycle parameters between control and exposed females (Table N10). The absolute and relative right epididymis and right testis weights of the 7,000 ppm F_1 males were significantly lower than those of the controls (Table N9), but the sperm counts were similar to that of the controls despite the lower epididymis and testis weights (Table N10). However, the percent abnormal sperm in the 7,000 ppm F_1 males was significantly greater than that in the controls. Incidences of lesions in the testis and epididymis were similar between control and 7,000 ppm males (Table N11).

In summary, phenolphthalein at concentrations up to 30,000 ppm produced significant reproductive toxicity in the absence of changes in body or somatic organ weights. While the second generation did not appear markedly more affected than the first, the pattern of effect was qualitatively different. F_0 males had functioning spermatogenesis at the start of the study. Toxicant-induced decreases in this process would translate into reduced sperm counts and testicular and epididymal weights, and these effects were observed in this study. In developing F_1 males, the toxic effect appeared as a larger decrease in testis weights (50% in F_1 males vs. 35% in F_0 males) with no significant decrease in sperm count and with no significant active lesions present in the seminiferous tubule epithelium. This pattern of response suggests an effect on the development of the testis, which would be consistent with an estrogenic effect, an anti-androgenic effect, or a thyroid gland effect. An anti-androgenic effect would not explain the effects on female reproduction that were observed, so the more likely explanation may be an estrogenic effect and/or interference with thyroid hormone economy. Additional studies are necessary to explore these effects.

TABLE N1
Fertility, Reproductive Performance, Length of Gestation, and Body Weight Data for F_0 and F_1 Swiss (CD-1®) Mice in the Continuous Breeding Study of Phenolphthalein[a]

	0 ppm	1,000 ppm	7,000 ppm	30,000 ppm
F_0 Adult Data				
Pregnancy index[b]				
Litter 1	38/38 (100%)	18/18 (100%)	19/19 (100%)	17/19 (89%)*
Litter 2	38/38 (100%)	18/18 (100%)	17/19 (89%)*	14/19 (74%)*
Litter 3	38/38 (100%)	18/18 (100%)	16/19 (84%)*	10/19 (53%)*
Litter 4	38/38 (100%)	18/18 (100%)	13/19 (68%)*	4/19 (21%)*
Litter 5	34/38 (89%)	18/18 (100%)	5/19 (26%)*	1/19 (5%)*
Average litters/pair	4.9 ± 0.1	5.0 ± 0.0	3.7 ± 0.3*	2.7 ± 0.3*
Cumulative days to litter				
Litter 1	22.3 ± 0.7	21.4 ± 0.3	29.7 ± 5.8	24.9 ± 2.0
Litter 2	42.4 ± 0.7	41.2 ± 0.3	47.9 ± 3.1*	51.1 ± 3.3*
Litter 3	63.3 ± 1.0	61.7 ± 0.5	74.8 ± 3.5*	76.2 ± 5.0*
Litter 4	84.3 ± 1.2	82.8 ± 0.9	94.7 ± 3.6*	93.5 ± 3.9*
Litter 5	103.2 ± 0.7	103.9 ± 1.0	101.8 ± 0.8	113.0[c]
Dam weight during lactation of litter 5 (g)				
n	37	18	14	4
Lactation day 0	48.2 ± 0.8	47.7 ± 1.0	45.3 ± 1.0	43.8 ± 0.9*
Lactation day 4	50.4 ± 0.9	49.0 ± 1.0	43.9 ± 1.2*	40.8 ± 0.4*
Lactation day 7	53.0 ± 0.7	51.7 ± 1.3	44.0 ± 1.7*	41.6 ± 1.5*
Lactation day 14	54.6 ± 0.9	53.2 ± 1.6	44.4 ± 1.9*	43.7 ± 1.3*
Lactation day 21	44.8 ± 0.9[d]	43.4 ± 1.5[e]	40.2 ± 1.5*[f]	41.9 ± 0.7
F_1 Pup Data (Litters 1 Through 5)				
Number of litters	38	18	17	17
Live male pups/litter	6.5 ± 0.2	6.2 ± 0.4	2.8 ± 0.5*[i]	2.5 ± 0.4*
Live female pups/litter	6.5 ± 0.2	5.9 ± 0.4	2.9 ± 0.4*[i]	2.9 ± 0.4*
Total live pups/litter	13.0 ± 0.3	12.1 ± 0.7	5.7 ± 0.9*[i]	5.4 ± 0.6*
Live pups/litter (%)	99 ± 0	97 ± 2	83 ± 7[i]	93 ± 4
Sex ratio[g] (%)	50 ± 1	51 ± 1	49 ± 3	48 ± 5
Male pup weight (g)	1.62 ± 0.01	1.61 ± 0.03	1.75 ± 0.05	1.62 ± 0.04[e]
Female pup weight (g)	1.56 ± 0.01	1.53 ± 0.03	1.65 ± 0.03	1.56 ± 0.04
Total live pup weight (g)	1.59 ± 0.01	1.57 ± 0.03	1.70 ± 0.04	1.60 ± 0.04
Adjusted total pup weight[h] (g)	1.66 ± 0.02	1.62 ± 0.03	1.61 ± 0.03	1.49 ± 0.04*

* Significantly different ($P \leq 0.05$) from the control group by a chi-square test (pregnancy indices), Shirley's test (average litters/pair, cumulative days to litter, dam weights, and live pups/litter), or Dunnett's test (adjusted pup weights)

[a] Data for average litters/pair, cumulative days to litter, body weights, live pups/litter, and sex ratio are given as mean ± standard error. Differences from the control group for sex ratio and nonadjusted pup weights are not significant by Dunn's test.

[b] Pregnant females/cohabiting pairs

[c] n=1; no standard error calculated

[d] n=36

[e] n=16

[f] n=13

[g] Live male pups/live pups

[h] Least-squares estimate of the mean of the average pup weight adjusted for average litter size

[i] n=19

TABLE N2
Litter and Body Weight Data for F_1 Swiss (CD-1®) Mouse Pups in the Continuous Breeding Study of Phenolphthalein[a]

	0 ppm	1,000 ppm	7,000 ppm	30,000 ppm
Litter 1				
Number of pairs delivering	38	17	15	15
Live pups/litter[b]	12.2 ± 0.5	10.7 ± 1.0[e]	6.4 ± 1.1*[f]	8.1 ± 1.2*[g]
Total live pup weight[c] (g)	1.60 ± 0.02	1.64 ± 0.06	1.72 ± 0.06	1.63 ± 0.05
Adjusted total live pup weight[d] (g)	1.66 ± 0.02	1.66 ± 0.03	1.62 ± 0.03	1.56 ± 0.03
Litter 2				
Number of pairs delivering	38	18	16	14
Live pups/litter	13.4 ± 0.5	12.2 ± 1.0	6.9 ± 1.1*[g]	5.0 ± 0.8*
Total live pup weight (g)	1.59 ± 0.02	1.56 ± 0.04	1.71 ± 0.05	1.63 ± 0.06
Adjusted total live pup weight (g)	1.66 ± 0.03	1.62 ± 0.04	1.61 ± 0.04	1.46 ± 0.05*
Litter 3				
Number of pairs delivering	38	18	15	8
Live pups/litter	13.6 ± 0.5	12.6 ± 1.0	5.1 ± 1.0*[h]	3.4 ± 1.1*[i]
Total live pup weight (g)	1.59 ± 0.02	1.58 ± 0.04	1.75 ± 0.05*	1.48 ± 0.08
Adjusted total live pup weight (g)	1.67 ± 0.02	1.63 ± 0.03	1.59 ± 0.04	1.30 ± 0.05*
Litter 4				
Number of pairs delivering	38	18	12	4
Live pups/litter	13.2 ± 0.5	12.9 ± 1.0	5.7 ± 1.0*[j]	3.8 ± 1.9*
Total live pup weight (g)	1.61 ± 0.02	1.57 ± 0.04	1.68 ± 0.05	1.48 ± 0.14
Adjusted total live pup weight (g)	1.66 ± 0.02	1.61 ± 0.03	1.53 ± 0.05	1.27 ± 0.08*
Litter 5				
Number of pairs delivering	34	18	5	1
Live pups/litter	12.6 ± 0.6	12.4 ± 0.8	10.2 ± 0.6*	2.0[k]
Total live pup weight (g)	1.66 ± 0.03	1.62 ± 0.03	1.59 ± 0.07	1.66[k]
Adjusted total live pup weight (g)	1.68 ± 0.02	1.62 ± 0.03	1.53 ± 0.06	1.36 ± 0.14
Litters 1 Through 5				
Number of pairs delivering	38	18	17	17
Live pups/litter	13.0 ± 0.3	12.1 ± 0.7	5.7 ± 0.9*[f]	5.4 ± 0.6*
Total live pup weight (g)	1.59 ± 0.01	1.57 ± 0.03	1.70 ± 0.04	1.60 ± 0.04
Adjusted total live pup weight (g)	1.66 ± 0.02	1.62 ± 0.03	1.61 ± 0.03	1.49 ± 0.04*

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test (live pups/litter and total live pup weights) or Dunnett's test (adjusted pup weights)
[a] Data are given as mean ± standard error.
[b] Mean of average number of live pups per litter for each fertile pair
[c] Mean of average live pup weight for each fertile pair
[d] Least-squares estimate of mean pup weight adjusted for average litter size
[e] n=18
[f] n=19
[g] n=17
[h] n=16
[i] n=10
[j] n=13
[k] n=1; no standard error calculated

TABLE N3
Survival and Body Weights of F_1 Swiss (CD-1®) Mouse Pups (Final Litter) in the Continuous Breeding Study of Phenolphthalein[a]

	0 ppm	1,000 ppm	7,000 ppm	30,000 ppm
Day 0				
Number of litters	37[b]	18	12[c]	3[d]
Male pup weight (g)	1.67 ± 0.03	1.65 ± 0.03	1.65 ± 0.05	1.50 ± 0.20
Female pup weight (g)	1.63 ± 0.03	1.59 ± 0.03	1.63 ± 0.04	1.49 ± 0.09
Day 4				
Male survival (%)	99 ± 1	98 ± 1	88 ± 13	29 ± 29*
Female survival (%)	97 ± 1	91 ± 6	75 ± 13	67 ± 33
Total survival (%)	98 ± 1	95 ± 2	75 ± 13	56 ± 29
Male pup weight (g)	3.34 ± 0.08	3.37 ± 0.09	3.28 ± 0.25[e]	1.89[f]
Female pup weight (g)	3.29 ± 0.09	3.28 ± 0.10[g]	3.02 ± 0.24[h]	2.39 ± 0.65
Day 7				
Male survival (%)	99 ± 1	98 ± 1	75 ± 16	29 ± 29*
Female survival (%)	97 ± 1	91 ± 6	67 ± 14	67 ± 33
Total survival (%)	98 ± 1	95 ± 2	67 ± 14	56 ± 29*
Male pup weight (g)	5.05 ± 0.13[b]	5.09 ± 0.14	5.53 ± 0.23[i]	3.06[f]
Female pup weight (g)	4.96 ± 0.16	4.93 ± 0.14[g]	4.93 ± 0.36[j]	3.92 ± 1.03[k]
Day 14				
Male survival (%)	99 ± 1	98 ± 1	75 ± 16	29 ± 29*
Female survival (%)	97 ± 1	90 ± 6	67 ± 14	67 ± 33
Total survival (%)	98 ± 1	95 ± 2	67 ± 14	56 ± 29*
Male pup weight (g)	7.68 ± 0.22	7.88 ± 0.39	9.18 ± 0.56*[i]	6.35[f]
Female pup weight (g)	7.72 ± 0.30	7.52 ± 0.30[g]	8.68 ± 0.47[j]	8.13 ± 1.97[k]
Day 21				
Male survival (%)	97 ± 1	92 ± 6	75 ± 16	29 ± 29*
Female survival (%)	96 ± 2	84 ± 8	67 ± 14	67 ± 33
Total survival (%)	97 ± 1	89 ± 6	67 ± 14	56 ± 29
Male pup weight (g)	11.98 ± 0.41[l]	12.84 ± 0.74[g]	14.16 ± 0.64*[i]	8.02[f]
Female pup weight (g)	11.49 ± 0.41	11.76 ± 0.54[m]	12.54 ± 0.60[j]	10.43 ± 3.49[k]

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test
[a] Data are given as mean ± standard error.
[b] Because no live male pups were born in one litter, n=36 for male pup survival and body weights.
[c] Because no live male pups were born in four litters, n=8 for male pup survival and body weights.
[d] Because no live male pups were born in one litter, n=2 for male pup survival and body weights.
[e] n=7
[f] n=1; no standard error calculated
[g] n=17
[h] n=9
[i] n=6
[j] n=8
[k] n=2
[l] n=35
[m] n=16

TABLE N4
Fertility, Reproductive Performance, Length of Gestation, and Body Weight Data for F_0 and F_1 Swiss (CD-1®) Mice in the Crossover Mating Trial of Phenolphthalein[a]

Male Exposure Group Female Exposure Group	0 ppm 0 ppm	7,000 ppm 0 ppm	0 ppm 7,000 ppm
F_0 Adult Data			
Mating index[b]	14/18 (78%)	18/19 (95%)	13/18 (72%)
Pregnancy index[c]	11/18 (61%)	14/19 (74%)	10/18 (56%)
Fertility index[d]	11/14 (79%)	14/18 (78%)	10/13 (77%)
Average dam weight at delivery (g)	46.8 ± 1.0	47.5 ± 1.0	41.7 ± 1.0*
Days to litter	19.0 ± 0.0	19.6 ± 0.2	19.9 ± 0.2*
F_1 Pup Data			
Number of litters	11	14	10
Live male pups/litter	6.0 ± 0.7	6.3 ± 0.9	2.3 ± 0.5*
Live female pups/litter	6.0 ± 0.8	4.7 ± 0.6	3.2 ± 0.7*
Total live pups/litter	12.0 ± 1.0	11.0 ± 1.3	5.5 ± 1.2*
Total live pups/litter (%)	100 ± 0	94 ± 6	85 ± 11
Sex ratio[e] (%)	51 ± 5	52 ± 6	38 ± 5[g]
Male pup weight (g)	1.67 ± 0.04	1.69 ± 0.05[h]	1.81 ± 0.10[i]
Female pup weight (g)	1.60 ± 0.03	1.63 ± 0.06	1.74 ± 0.07[g]
Total live pup weight (g)	1.64 ± 0.03	1.67 ± 0.05	1.77 ± 0.08[g]
Adjusted total live pup weight[f] (g)	1.70 ± 0.04	1.71 ± 0.04	1.63 ± 0.05[g]

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

[a] Data for body weights, days to litter, live pups/litter, and sex ratio are given as mean ± standard error. Differences from the control group for mating, pregnancy, and fertility indices were not significant by a chi-square test; differences for sex ratio and pup weights were not significant by Dunn's test; and differences for adjusted pup weights were not significant by a *t*-test.

[b] Females with sperm plug/cohabiting pairs

[c] Pregnant females/cohabiting pairs

[d] Pregnant females/females with sperm plug

[e] Live male pups/live pups

[f] Least-squares estimate of the mean pup weight adjusted for average litter size

[g] n=9

[h] n=13

[i] n=8

TABLE N5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_0 Swiss (CD-1®) Mice Administered Phenolphthalein in Feed[a]

	0 ppm	7,000 ppm
Male		
n	39	20
Necropsy body wt	42.4 ± 0.71	42.0 ± 0.95
R. Cauda Epididymis		
Absolute	18.0 ± 0.37	17.7 ± 0.79
Relative	0.43 ± 0.01	0.42 ± 0.02
R. Epididymis		
Absolute	56.6 ± 0.98	37.5 ± 3.5*
Relative	1.3 ± 0.03	0.91 ± 0.09*
Kidneys and Adrenal Glands		
Absolute	834.0 ± 12.6	870.7 ± 17.2*
Relative	19.8 ± 0.36	20.9 ± 0.64
Liver		
Absolute	2.2 ± 0.04	2.3 ± 0.06
Relative	51.8 ± 0.76	54.1 ± 0.69
Prostate Gland		
Absolute	25.0 ± 1.1	25.5 ± 1.4
Relative	0.59 ± 0.03	0.61 ± 0.04
Seminal Vesicles		
Absolute	692.1 ± 18.0	686.3 ± 20.4
Relative	16.4 ± 0.37	16.4 ± 0.43
R. Testis		
Absolute	142.2 ± 3.1	93.3 ± 4.6*
Relative	3.4 ± 0.08	2.2 ± 0.12*
Female		
n	37	18
Necropsy body wt	40.5 ± 0.70	39.0 ± 0.66
Kidneys and Adrenal Glands		
Absolute	645.7 ± 13.1	634.6 ± 20.3
Relative	16.0 ± 0.28	16.3 ± 0.43
Liver		
Absolute	2.3 ± 0.05	2.3 ± 0.06
Relative	57.3 ± 0.87	57.9 ± 1.1
R. Ovary		
Absolute	11.3 ± 0.44	11.2 ± 0.65
Relative	0.28 ± 0.01	0.29 ± 0.02

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

TABLE N6
Sperm Parameters and Estrous Cycle Characterization for F_0 Swiss (CD-1®) Mice Administered Phenolphthalein in Feed[a]

	0 ppm	7,000 ppm
Male		
n	39	20
Epididymal spermatozoal parameters		
Motility (%)	91.0 ± 0.6	86.7 ± 2.8[b]
Abnormal (%)	2.6 ± 0.3	4.1 ± 0.5*[c]
Concentration (10^6/g cauda epididymal tissue)	1,167 ± 37	816 ± 88*
Female		
n	37	18
Estrous cycle length (days)	4.92 ± 0.12[d]	4.68 ± 0.17[e]
Estrous stages (% of cycle)		
Diestrus	47.1	45.8
Proestrus	14.2	14.4
Estrus	25.7	26.4
Metestrus	13.1	13.4

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

[a] Epididymal spermatozoal parameters and estrous cycle lengths are given as mean ± standard error. Differences from the control group for epididymal spermatozoal motility are not significant by Wilcoxon's test. By multivariate analysis of variance, exposed females do not differ significantly from control females in cycle length or in the relative length of time spent in estrous stages.

[b] n=19

[c] n=18

[d] Estrous cycle length was longer than 7 days or was unclear in 7 of 37 animals.

[e] Estrous cycle length was longer than 7 days or was unclear in 4 of 18 animals.

TABLE N7
Incidences of Selected Nonneoplastic Lesions in Male F_0 Swiss (CD-1®) Mice Administered Phenolphthalein in Feed

	0 ppm	7,000 ppm
Testis[a]	11	10
Seminiferous Tubule, Degeneration[b]	2 (2.0)[c]	9** (1.9)
Interstitial Cell Hyperplasia	0	4* (1.3)
Epididymis	11	10
Epithelial Cell, Degeneration	5 (1.0)	7 (1.1)
Presence of Fluid/Degenerated Cell	1 (4.0)	7** (1.3)

* 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 = severe

TABLE N8
Fertility, Reproductive Performance, Length of Gestation, and Body Weight Data for F_1 and F_2 Swiss (CD-1®) Mice in the Offspring Assessment Phase of the Continuous Breeding Study of Phenolphthalein[a]

	0 ppm	1,000 ppm	7,000 ppm
F_1 Adult Data			
Mating index[b]	19/20 (95%)	20/20 (100%)	19/20 (95%)
Pregnancy index[c]	17/20 (85%)	17/20 (85%)	9/20 (45%)*
Fertility index[d]	17/19 (89%)	17/20 (85%)	9/19 (47%)
Dam weight at delivery (g)	35.9 ± 0.8	37.1 ± 0.9	34.8 ± 0.8
Days to litter	19.1 ± 0.2[e]	19.0 ± 0.1	19.7 ± 0.2*
F_2 Pup Data			
Number of litters	17	17	9
Live male pups/litter	4.7 ± 0.6	5.5 ± 0.5	1.6 ± 0.4*
Live female pups/litter	4.5 ± 0.5	6.3 ± 0.6	3.3 ± 0.4
Total live pups/litter	9.2 ± 1.0	11.8 ± 0.5	4.9 ± 0.8*
Total live pups/litter (%)	94 ± 6	100 ± 0	100 ± 0
Sex ratio[f] (%)	47 ± 5[e]	47 ± 4	25 ± 7*
Male pup weight (g)	1.67 ± 0.03[h]	1.60 ± 0.03	1.88 ± 0.05*[i]
Female pup weight (g)	1.61 ± 0.04[e]	1.51 ± 0.03	1.76 ± 0.04*
Total live pup weight (g)	1.66 ± 0.04[e]	1.55 ± 0.03	1.80 ± 0.03*
Adjusted total live pup weight[g] (g)	1.66 ± 0.03[e]	1.61 ± 0.03	1.67 ± 0.05

* Significantly different ($P \leq 0.05$) from the control group by a chi-square test (pregnancy index) or Dunn's or Shirley's test (days to litter, live pups/litter, sex ratio, and pup weights)

[a] Data for body weights, days to litter, live pups/litter, and sex ratio are given as mean ± standard error. Differences from the control group for mating and fertility indices are not significant by a chi-square test, differences for dam weights were not significant by Dunn's test, and differences for adjusted pup weights are not significant by Dunnett's test.

[b] Females with sperm plug/cohabiting pairs

[c] Pregnant females/cohabiting pairs

[d] Pregnant females/females with sperm plug

[e] n=16

[f] Live male pups/live pups

[g] Least-squares estimate of mean pup weight adjusted for average litter size

[h] n=15

[i] n=6

TABLE N9
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1 Swiss (CD-1®) Mice in the Offspring Assessment Phase of the Continuous Breeding Study of Phenolphthalein[a]

	0 ppm	1,000 ppm	7,000 ppm
n	20	20	20
Male			
Necropsy body wt	34.9 ± 0.73	34.7 ± 0.49	33.4 ± 0.44
R. Cauda Epididymis			
Absolute	14.7 ± 0.55	15.2 ± 0.50	13.2 ± 0.53
Relative	0.42 ± 0.02	0.44 ± 0.02	0.40 ± 0.02
R. Epididymis			
Absolute	45.1 ± 1.5	47.6 ± 0.96	37.4 ± 1.4*
Relative	1.3 ± 0.05	1.4 ± 0.03	1.1 ± 0.03*
Kidneys and Adrenal Glands			
Absolute	723.3 ± 20.5	723.1 ± 18.8	709.6 ± 13.3
Relative	20.8 ± 0.50	20.9 ± 0.50	21.3 ± 0.41
Liver			
Absolute	1.8 ± 0.05	1.9 ± 0.05	1.8 ± 0.04
Relative	51.6 ± 1.0	54.0 ± 1.0	53.8 ± 1.0
Prostate Gland			
Absolute	19.9 ± 1.3[b]	18.1 ± 1.5	16.0 ± 1.2[b]
Relative	0.57 ± 0.03[b]	0.52 ± 0.04	0.48 ± 0.03[b]
Seminal Vesicles			
Absolute	400.4 ± 12.2	387.9 ± 13.5	398.2 ± 14.3
Relative	11.5 ± 0.32	11.2 ± 0.38	11.9 ± 0.41
R. Testis			
Absolute	134.7 ± 6.2	139.5 ± 4.7	74.5 ± 5.5*
Relative	3.9 ± 0.18	4.0 ± 0.14	2.2 ± 0.15*
Female			
Necropsy body wt	30.9 ± 0.61	30.7 ± 0.67	29.0 ± 0.55*
Kidneys and Adrenal Glands			
Absolute	514.7 ± 23.4	478.3 ± 10.5	489.2 ± 9.3
Relative	16.7 ± 0.67	15.6 ± 0.24	17.0 ± 0.38
Liver			
Absolute	1.8 ± 0.08	1.7 ± 0.04	1.6 ± 0.04*
Relative	57.5 ± 2.1	56.2 ± 0.99	54.8 ± 1.4
R. Ovary			
Absolute	9.1 ± 0.60[b]	7.9 ± 0.53	8.1 ± 0.50
Relative	0.30 ± 0.02[b]	0.26 ± 0.02	0.28 ± 0.02

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test
[a] Liver weights and body weights are given in grams; other organ weights are given in milligrams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=19

TABLE N10
Sperm Parameters and Estrous Cycle Characterization for F_1 Swiss (CD-1®) Mice in the Offspring Assessment Phase of the Continuous Breeding Study of Phenolphthalein[a]

	0 ppm	1,000 ppm	7,000 ppm
Male			
n	20	20	20
Epididymal spermatozoal parameters			
Motility (%)	79.4 ± 5.5	85.3 ± 3.6	83.3 ± 4.7
Abnormal (%)	2.2 ± 0.3	2.4 ± 0.2	4.9 ± 0.8*
Concentration (10^6/g cauda epididymal tissue)	970 ± 85	1,087 ± 44	772 ± 86
Female			
n	20	20	20
Estrous cycle length (days)	4.90 ± 0.13	4.84 ± 0.14[b]	5.03 ± 0.12[c]
Estrous stages (% of cycle)			
Diestrus	25.4	22.1	24.2
Proestrus	18.3	18.3	16.7
Estrus	39.2	42.9	45.8
Metestrus	16.7	16.2	12.9
Uncertain diagnoses	0.4	0.4	0.4

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

[a] Epididymal spermatozoal parameters and estrous cycle lengths are given as mean ± standard error. Differences from the control group for epididymal spermatozoal motility and concentration are not significant by Dunn's test. By multivariate analysis of variance, exposed females do not differ significantly from control females in cycle length or in the relative length of time spent in estrous stages.

[b] Estrous cycle length was longer than 7 days or was unclear in 1 of 20 animals.

[c] Estrous cycle length was longer than 7 days or was unclear in 3 of 20 animals.

TABLE N11
Incidences of Selected Nonneoplastic Lesions in Male F_1 Swiss (CD-1®) Mice in the Offspring Assessment Phase of the Continuous Breeding Study of Phenolphthalein

	0 ppm	7,000 ppm
Testis[a]	10	10
Seminiferous Tubule, Degeneration[b]	5 (1.2)[c]	7 (1.3)
Interstitial Cell Hyperplasia	0	1 (2.0)
Epididymis	10	10
Epithelial Cell, Degeneration	4 (1.3)	6 (1.2)
Presence of Fluid/Degenerated Cell	1 (1.0)	1 (2.0)

[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 = severe

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

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

INTRODUCTION

Phenolphthalein is an isobenzofuranone derivative that is used as a cathartic and laxative. The single-dose toxicokinetic studies conducted in F344/N rats and B6C3F$_1$ mice were performed to determine the pharmacokinetic profile and elimination kinetics of phenolphthalein in rats and mice following a single dose by gavage (NTP, 1994).

MATERIALS AND METHODS

Phenolphthalein was obtained from Pharmco Laboratories, Inc. (Titusville, FL) in one lot (P9189-J1), which was also used for the 2-year studies conducted at TSI Mason Laboratories (Worcester, MA). Results of purity and stability analyses of lot P9189-J1 are given in Appendix J. Dose formulations were prepared as needed by suspending appropriate amounts of phenolphthalein in 0.5% aqueous methylcellulose, and analyses by the study laboratory using high-performance liquid chromatography (HPLC) indicated that the dose formulations were homogeneous and within 10.7% of the target concentrations. Stability studies of a 5 mg/mL dose formulation conducted by the study laboratory indicated that suspensions are stable for up to 14 days when stored at room temperature and protected from light in sealed vials.

F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). Upon receipt, the rats and mice were observed for parasites and indications of disease. The rats were quarantined for 140 (males) or 141 (females) days and were 171 (males) or 172 (females) days old at the start of the studies. The mice were quarantined for 145 (males) or 146 (females) days and were 177 (males) or 178 (females) days old at the start of the studies. Filtered municipal water and NIH-07 open formula meal diet were available *ad libitum*. During the studies, the rats administered 100 mg phenolphthalein/kg body weight were housed three per cage, and the rats administered 250 mg/kg were housed two per cage. The mice were housed separately.

Doses and sampling time points for the single-dose toxicokinetic studies were selected based on analysis of data from preliminary toxicokinetic studies and in preparation for the 2-year toxicokinetic studies.

Groups of 30 male and 30 female rats were administered a single dose of 100 mg phenolphthalein/kg body weight by gavage, and groups of 14 male and 16 female rats were administered 250 mg/kg. The dosing volume was 5 mL/kg. Groups of 30 male and 30 female mice were administered 50 mg/kg, and groups of 14 male and 16 female mice were administered 125 mg/kg. The dosing volume was 10 mL/kg. The animals were anesthetized with a mixture of carbon dioxide and oxygen, and blood samples were collected from the retroorbital sinus. Blood samples were collected from three male and three female 100 mg/kg rats and from three male and three female 50 mg/kg mice per time point at 5, 15, and 30 minutes and at 2, 4, 6, 10, 24, 34, and 48 hours after the administration of phenolphthalein. Blood samples were collected from two 250 mg/kg male rats and two 125 mg/kg male mice per time point at 30 minutes and at 3, 5, 8, 24, 34, and 48 hours after the administration of phenolphthalein; blood samples were collected from two 250 mg/kg female rats and two 125 mg/kg female mice per time point at 15 and 30 minutes and at 3, 5, 8, 24, 34, and 48 hours after the administration of phenolphthalein. Blood samples were collected only once from each animal. The samples were processed by the study laboratory, and the plasma was stored at −20° C or lower until analysis.

All animals were observed twice per day, 7 days per week. Clinical observations were not recorded for these studies. Individual body weights were recorded on the first day of the studies for the calculation of dosing volumes.

A plasma analysis procedure was developed and evaluated at the study laboratory for the analysis of plasma concentrations of total phenolphthalein (free and conjugated) at concentrations ranging from 0.5 to 60 μg/mL. Concentrations less than the experimental level of quantitation (ELOQ=0.5 μg/mL) should be considered approximations. Plasma samples were treated with β-glucuronidase and sulfatase enzyme preparations. Solid-phase extraction with octadecyl packing material was used to isolate total phenolphthalein and clean up the extract. The samples were then analyzed using HPLC with a Whatman pellicular ODS guard column, a Waters μBondapak 10 μm C_{18} column, ultraviolet detection (229 nm), and a mobile phase of methanol:water:0.1 N hydrochloric acid (530:470:10) with a 1.0 mL/minute flow rate.

The average plasma concentrations of total phenolphthalein and standard deviations were calculated. The logarithms of these values were plotted as a function of time. The areas under the plasma concentration versus time curves (AUC_t) and standard errors were calculated using the trapezoid rule of the form $AUC_t = \sum\{(C_n+C_{n-1})/2\}\times\{t_n-t_{n-1}\}$, where AUC_t is the cumulative area under the curve to time t and C_{n-1} and C_n are successive concentrations at t_{n-1} and t_n, respectively. The areas under the curves to infinity (AUC_0^∞) were calculated from $AUC_0^\infty = AUC_t + C_t/\lambda$, where C_t is the last measured time point and λ is the terminal rate constant determined from the slope of the terminal phase of the log plasma concentration-time profiles. Linear regression of the last three or four data points gave the slope (λ). The half-lives ($t_{½}$) were calculated as $0.693/\lambda$. The total body clearance (Cl) was calculated as dose/AUC_0^∞. The clearance gives an estimate of the volumetric elimination of the drug from the body. The maximum observed concentration (C_{max}) and corresponding time (t_{max}) were determined from the plasma concentration-time data as the maximum observed concentration and corresponding time, respectively.

RESULTS

The plasma concentrations of total phenolphthalein and their standard deviations in rats are given in Table O1. The semilogarithmic plots of plasma concentration-time data for male and female rats administered 100 and 250 mg/kg are shown in Figures O1 through O4 and show linear kinetics in this dose range. The AUC_0^∞, $t_{½}$, Cl, C_{max} and t_{max} values for rats are reported in Table O2. The $t_{½}$ values were generally similar among groups. For rats administered 100 mg/kg, the $t_{½}$ values were 13.1 hours for males and 12.3 hours for females. For rats administered 250 mg/kg, the $t_{½}$ values were 13.8 hours for males and 28.3 hours for females. For rats administered 100 mg/kg, the Cl values were 0.157 mL/hour/kg for males and 0.118 mL/hour/kg for females. For rats administered 250 mg/kg, the Cl values were 0.286 mL/hour/kg for males and 0.133 mL/hour/kg for females. The C_{max} values were similar for the male and female rats administered 100 mg/kg. For rats administered 250 mg/kg, C_{max} values were 49.4 mg/kg for males and 63.4 mg/kg for females. The t_{max} values were generally similar for 100 and 250 mg/kg males and females and ranged from 2 to 5 hours.

The plasma concentrations of total phenolphthalein and their standard deviations in mice are given in Table O1. The semilogarithmic plots of plasma concentration-time data for male and female mice administered 50 and 125 mg/kg are shown in Figures O5 through O8 and show linear kinetics in this dose range. However, the scatter on the 125 mg/kg females did not allow for the accurate estimation of the terminal slope, and the slope in this case was determined between the 8- and 48-hour time points. The AUC_0^∞, $t_{½}$, Cl, C_{max} and t_{max} values for mice are reported in Table O3. The $t_{½}$ values were similar among groups. For mice administered 50 mg/kg, the $t_{½}$ values were 6.28 hours for males and 6.68 hours for females. For mice administered 125 mg/kg, the $t_{½}$ values were 6.52 hours for males and 6.96 hours for females. For mice administered 50 mg/kg, the Cl values were 0.104 mL/hour/kg for males and

0.074 mL/hour/kg for females. For mice administered 125 mg/kg, the Cl values were 0.168 mL/hour/kg for males and 0.119 mL/hour/kg for females. For mice administered 50 mg/kg, C_{max} values were 29.5 mg/kg for males and 44.5 mg/kg for females. For mice administered 125 mg/kg, C_{max} values were 50.7 mg/kg for males and 79.0 mg/kg for females. The t_{max} values demonstrated an erratic trend. The 50 mg/kg males showed a maximum at 4 hours, and the 125 mg/kg males showed a maximum at 30 minutes. The 50 mg/kg females showed a maximum at 30 minutes, and the 125 mg/kg females showed a maximum at 5 hours. This could be due to one or a combination of interanimal, assay, and dosing variabilities.

Statistical analysis of the data has not been performed; however, it appears that 1) the elimination half-lives were longer for rats than for mice; 2) the AUC_0^∞s were not dose proportional for either males or females or for rats or mice; and 3) the AUC_0^∞s were larger for female rats and mice than for males. Thus, these data indicate that females are exposed to higher plasma concentrations of total phenolphthalein and that some process, possibly absorption, is saturated at the high dose.

TABLE O1
Plasma Concentrations of Total Phenolphthalein in F344/N Rats and B6C3F$_1$ Mice After a Single Gavage Dose of Phenolphthalein[a]

	100 mg/kg Rats	250 mg/kg Rats	50 mg/kg Mice	125 mg/kg Mice
n	3	2	3	2
Male				
Time After Dosing (minutes)				
5	1.77 ± 0.85	—[b]	14.30 ± 1.97	—
15	6.67 ± 1.36	—	22.10 ± 10.14	—
30	12.21 ± 3.46	14.68 ± 7.53	25.10 ± 5.08	50.65 ± 0.64
120	27.90 ± 0.92	—	28.27 ± 3.92	—
180	—	39.00 ± 7.78	—	45.55 ± 4.17
240	33.27 ± 5.35	—	29.47 ± 7.73	—
300	—	49.40 ± 0.42	—	40.90 ± 1.56
360	29.17 ± 1.52	—	24.20 ± 3.00	—
480	—	29.45 ± 11.53	—	37.85 ± 2.62
600	20.27 ± 5.12	—	24.37 ± 3.96	—
1,440	8.37 ± 2.89	11.75 ± 1.34	2.70 ± 0.84	5.21 ± 0.16
2,040	6.12 ± 2.00	7.94 ± 2.18	1.35 ± 0.18	1.96 ± 0.63
2,880	2.57 ± 0.51	4.17 ± 2.64	0.33 ± 0.07[c]	0.61[d]
Female				
Time After Dosing (minutes)				
5	2.50 ± 0.10	—	16.47 ± 0.50	—
15	9.39 ± 1.53	10.83 ± 4.77	37.20 ± 6.49	67.25 ± 6.01
30	17.47 ± 5.31	26.20 ± 3.39	44.47 ± 8.82	71.85 ± 6.29
120	31.00 ± 2.46	—	44.20 ± 3.63	—
180	—	57.30 ± 11.17	—	74.50 ± 1.56
240	28.10 ± 7.14	—	32.00 ± 6.68	—
300	—	63.35 ± 25.67	—	79.00 ± 4.38
360	27.73 ± 4.65	—	40.23 ± 9.09	—
480	—	45.80[d]	—	47.40 ± 0.14
600	28.83 ± 1.12	—	34.60 ± 10.90	—
1,440	16.83 ± 3.16	16.55 ± 1.20	2.32 ± 1.01	3.00 ± 2.03
2,040	6.44 ± 2.00	11.80 ± 3.54	0.97 ± 0.15	5.95 ± 0.33
2,880	3.93 ± 1.27	15.93 ± 9.16	0.87 ± 0.51[e]	0.57 ± 0.08

[a] Data are given in μg/mL as mean ± standard deviation. Total phenolphthalein equals free and conjugated phenolphthalein.
[b] No samples were collected.
[c] All values were below the experimental limit of quantitation (0.5 μg/mL).
[d] n=1; no standard deviation calculated
[e] n=2

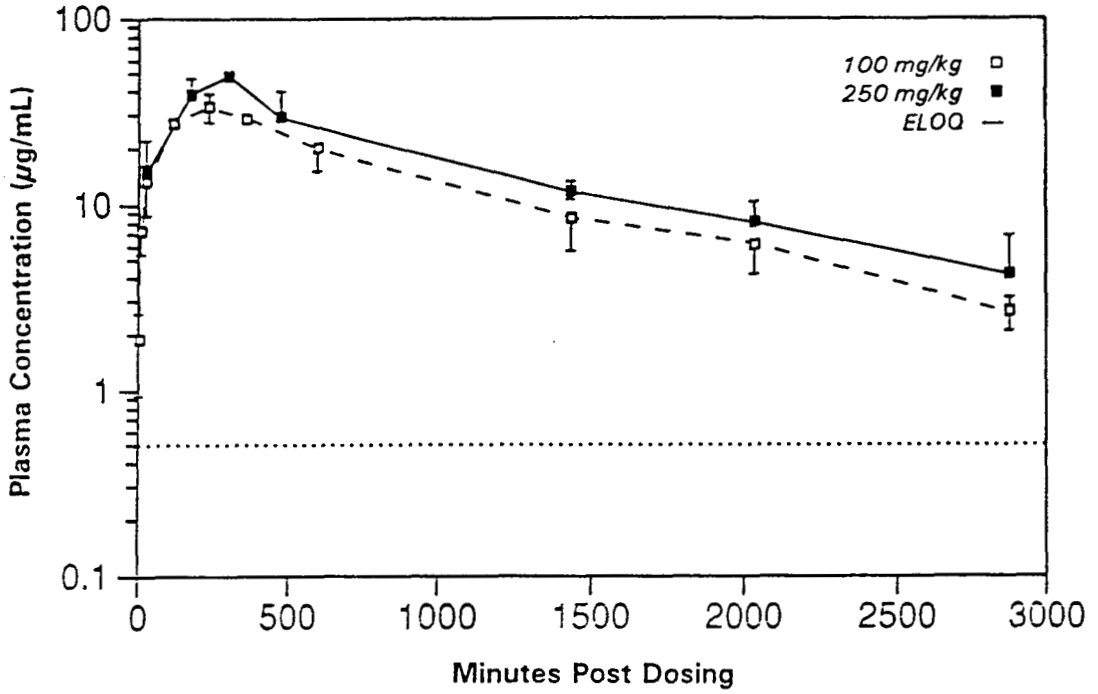

FIGURE O1
Plasma Concentrations of Total Phenolphthalein in Male F344/N Rats After a Single Gavage Dose of 100 or 250 mg/kg

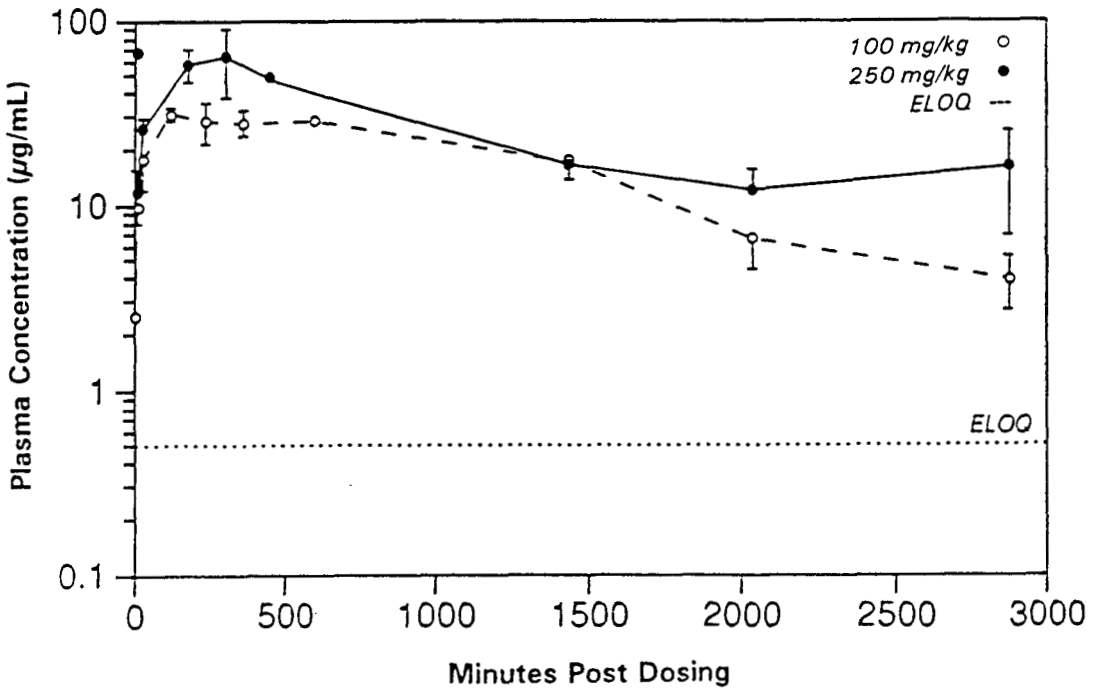

FIGURE O2
Plasma Concentrations of Total Phenolphthalein in Female F344/N Rats After a Single Gavage Dose of 100 or 250 mg/kg

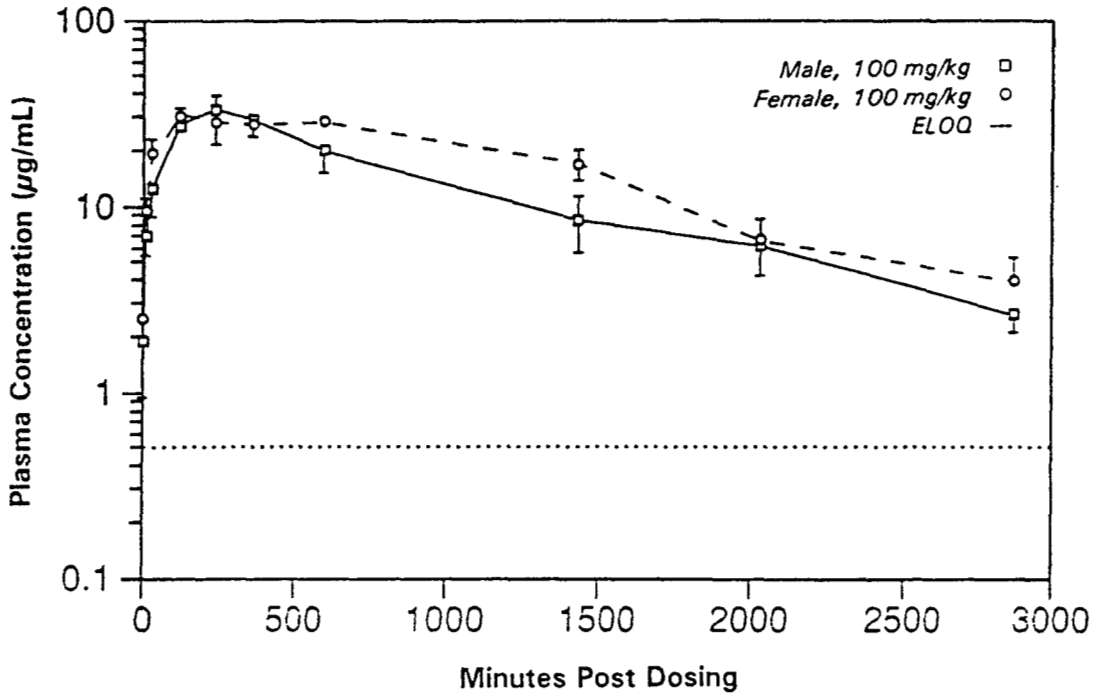

FIGURE O3
Plasma Concentrations of Total Phenolphthalein in Male and Female F344/N Rats After a Single Gavage Dose of 100 mg/kg

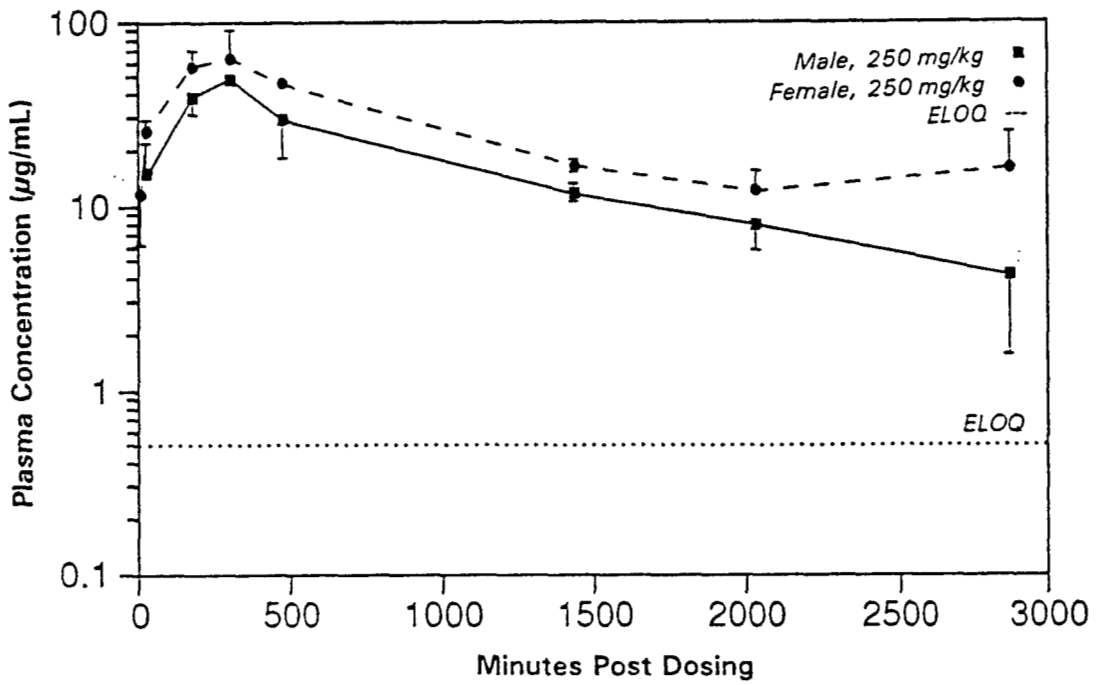

FIGURE O4
Plasma Concentrations of Total Phenolphthalein in Male and Female F344/N Rats After a Single Gavage Dose of 250 mg/kg

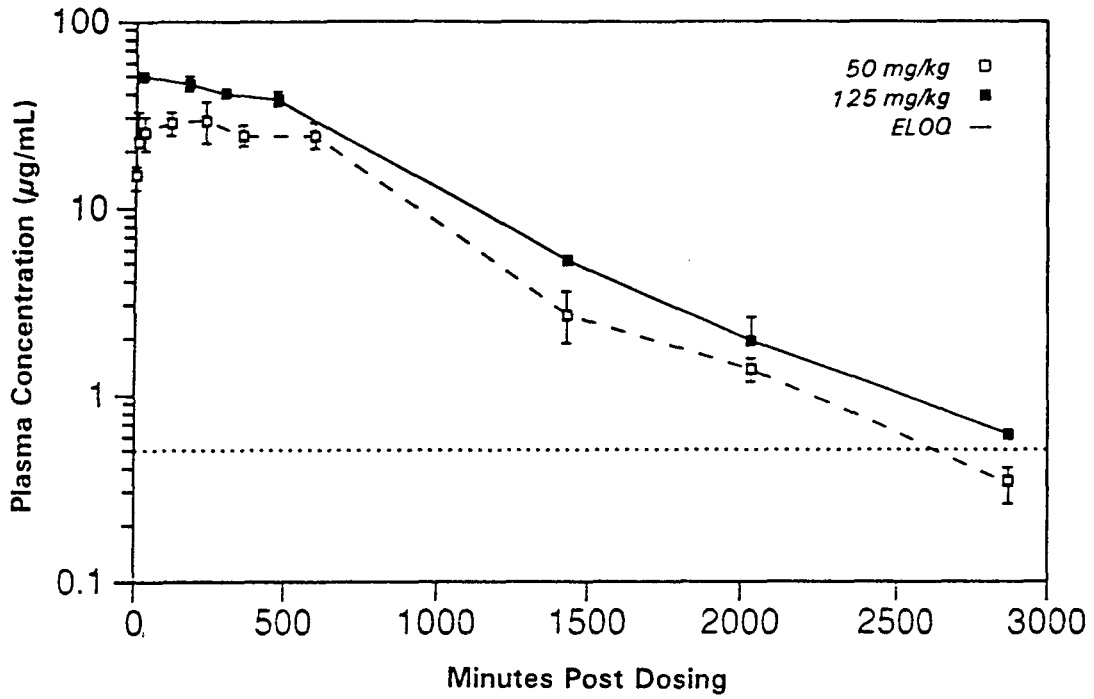

FIGURE O5
Plasma Concentrations of Total Phenolphthalein in Male B6C3F$_1$ Mice After a Single Gavage Dose of 50 or 125 mg/kg

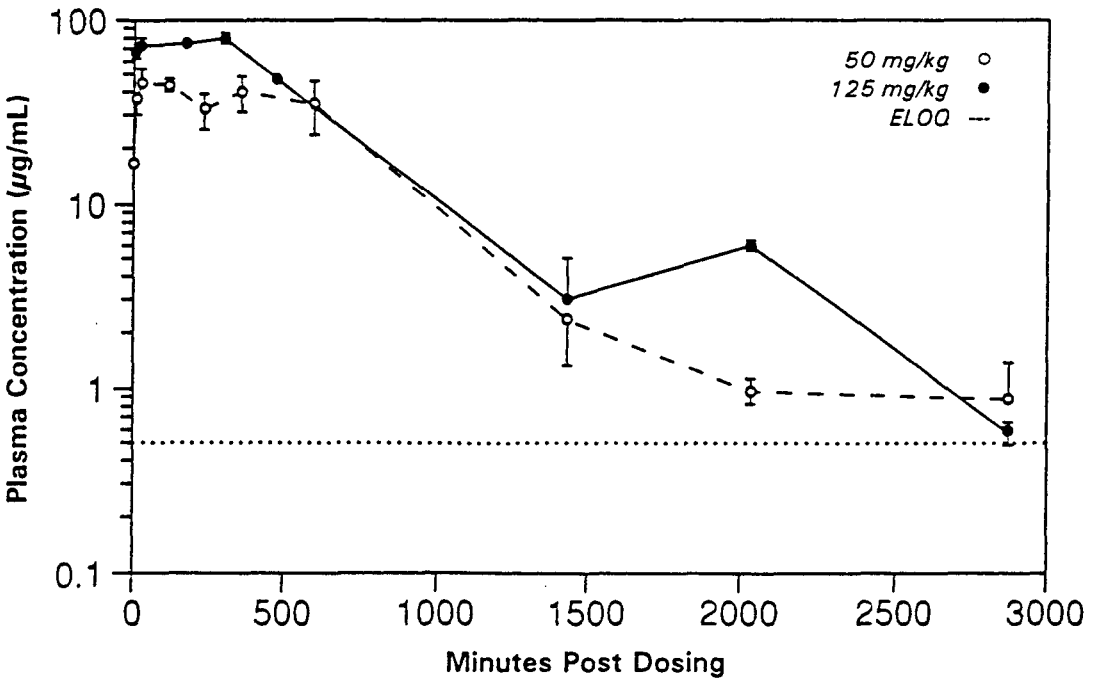

FIGURE O6
Plasma Concentrations of Total Phenolphthalein in Female B6C3F$_1$ Mice After a Single Gavage Dose of 50 or 125 mg/kg

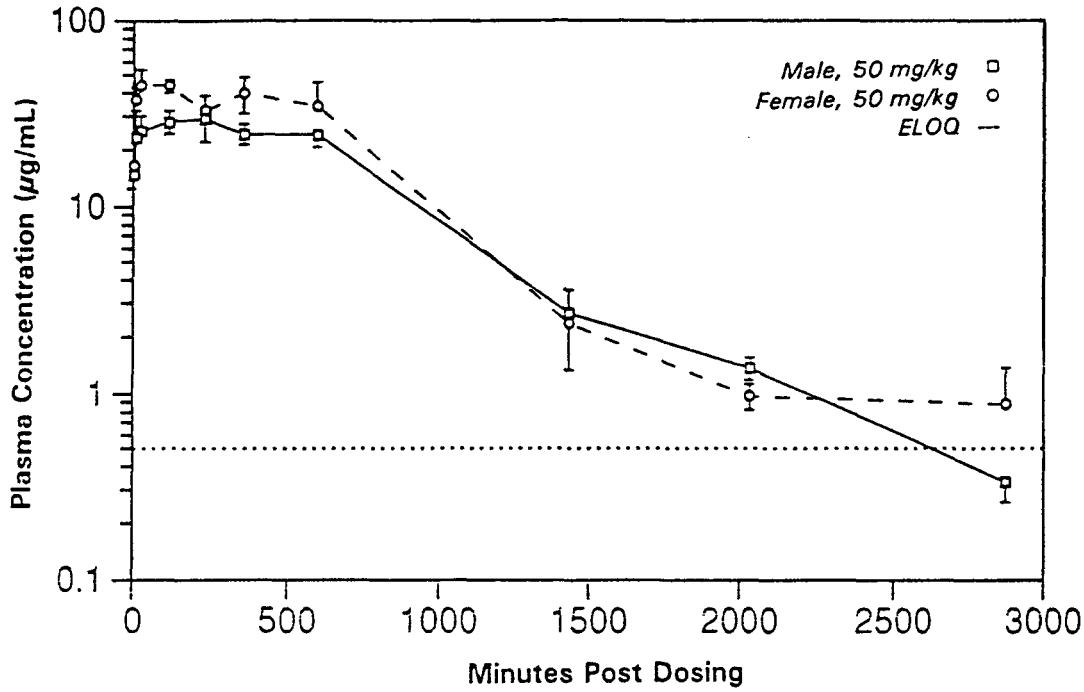

FIGURE O7
Plasma Concentrations of Total Phenolphthalein in Male and Female B6C3F$_1$ Mice After a Single Gavage Dose of 50 mg/kg

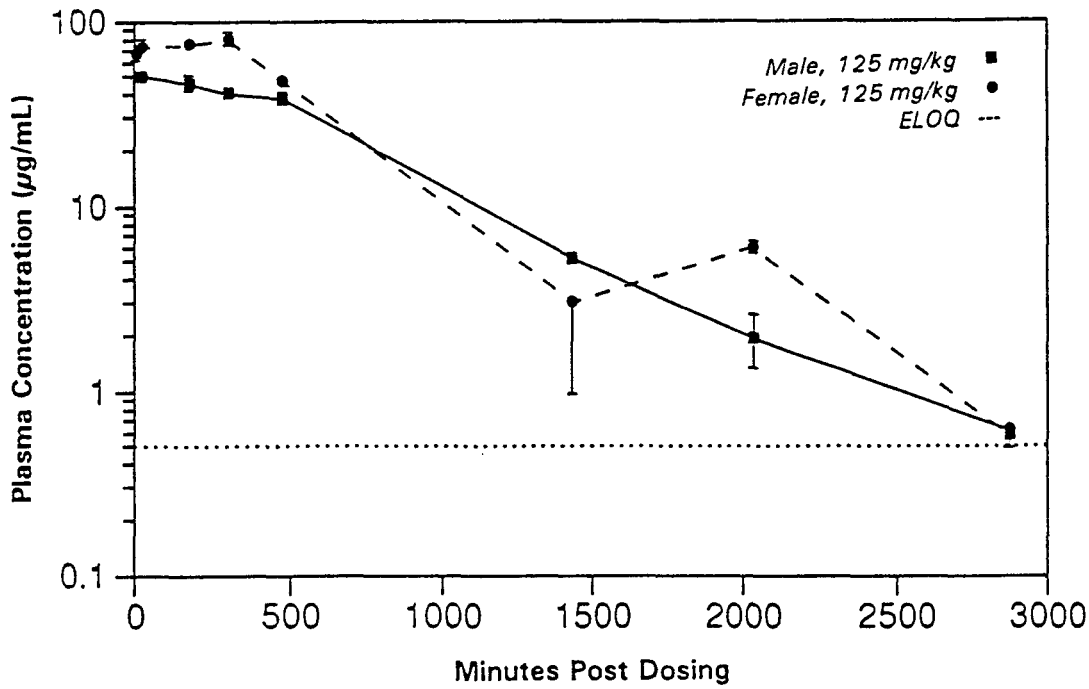

FIGURE O8
Plasma Concentrations of Total Phenolphthalein in Male and Female B6C3F$_1$ Mice After a Single Gavage Dose of 125 mg/kg

TABLE O2
Pharmacokinetic Parameters in F344/N Rats After a Single Gavage Dose of Phenolphthalein[a]

Dose (mg/kg)	AUC_0^{∞}[b] (μg•hour/mL)	$t_{½}$[c] (hours)	Cl[d] (mL/hour/kg)	C_{max}[e] (mg/kg)	t_{max}[f] (hours)
Male					
100	638 ± 38	13.1	0.157	33.3	4.00
250	873 ± 88	13.8	0.286	49.4	5.00
Female					
100	847 ± 32	12.3	0.118	31.0	2.00
250	1,879 ± 275	28.3	0.133	63.4	5.00

[a] The data were calculated from the plasma concentration-time curves, where each point represents the mean of three male or three female 100 mg/kg rats or of two male or two female 250 mg/kg rats.
[b] AUC_0^{∞} = area under the curve to infinity
[c] $t_{½}$ = elimination half-life
[d] Cl = Dose/AUC_0^{∞}
[e] C_{max} = maximum mean concentration
[f] t_{max} = time of maximum mean concentration (estimated from last four timepoints)

TABLE O3
Pharmacokinetic Parameters in B6C3F$_1$ Mice After a Single Gavage Dose of Phenolphthalein[a]

Dose (mg/kg)	AUC_0^{∞}[b] (μg•hour/mL)	$t½$[c] (hours)	Cl[d] (mL/hour/kg)	C_{max}[e] (mg/kg)	t_{max}[f] (hours)
Male					
50	482 ± 23	6.28	0.104	29.5	4.00
125	742 ± 18	6.52	0.168	50.7	0.50
Female					
50	676 ± 55	6.68	0.074	44.5	0.50
125	1,051 ± 18	6.96	0.119	79.0	5.00

[a] The data were calculated from the plasma concentration-time curves, where each point represents the mean of three male or three female 50 mg/kg mice or of two male or two female 125 mg/kg mice.
[b] AUC_0^{∞} = area under the curve to infinity
[c] $t_{½}$ = elimination half-life
[d] Cl = Dose/AUC_0^{∞}
[e] C_{max} = maximum mean concentration
[f] t_{max} = time of maximum mean concentration (estimated from last four timepoints)

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