NATIONAL TOXICOLOGY PROGRAM
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TOXICOLOGY AND CARCINOGENESIS STUDIES OF RHODAMINE 6G

(C.I. BASIC RED 1)

(CAS NO. 989-38-8)

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

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF RHODAMINE 6G

(C.I. BASIC RED 1)

(CAS NO. 989-38-8)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

John Edgar French, Ph.D., Study Scientist

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

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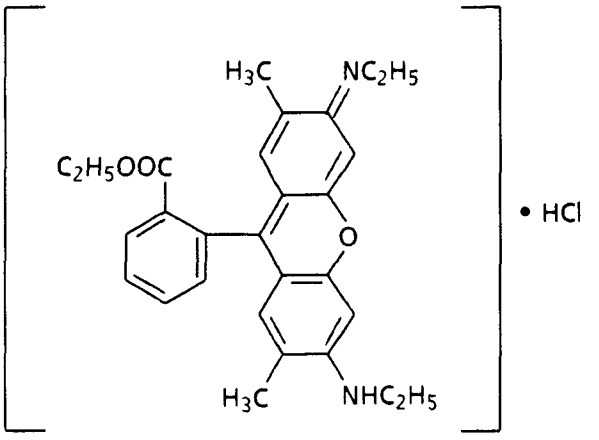
CONTENTS

APPENDIXES



RHODAMINE 6G

2-[6-(ethylamino)-3-(ethylimino)-2,7-dimethyl-3*H*-xanthen-9-yl] benzoic acid ethyl ester, monohydrochloride

CAS No. 989-38-8

$C_{28}H_{30}N_2O_3$•HCl Molecular weight 479.06

Common Names: Basic Red 1; Basic Rhodamine Yellow; Basic Rhodaminic Yellow; Calcozine Red 6G; Calcozine Rhodamine 6GX; C.I. Basic Red 1, Monohydrochloride; Elcozine Rhodamine 6GDN; Eljon Pink Toner; Fanal Pink GFK; Fanal Red 25532; Flexo Red 482; Heliostable Brilliant Pink B extra; Mitsui Rhodamine 6GCP; Nyco Liquid Red GF; Rhodamine 69DN Extra; Rhodamine F4G; Rhodamine F5G; Rhodamine F5G chloride; Rhodamine 6GB; Rhodamine 6GBN; Rhodamine 6GCP; Rhodamine 6GD; Rhodamine 4GD; Rhodamine GDN; Rhodamine 5GDN; Rhodamine 6 GDN; Rhodamine GDN Extra; Rhodamine 6GEx ethyl ester; Rhodamine 6G Extra; Rhodamine 6G Extra Base; Rhodamine 4GH; Rhodamine 6GH; Rhodamine 5GL; Rhodamine 6G lake; Rhodamine 6GX; Rhodamine J; Rhodamine 6JH; Rhodamine 7JH; Rhodamine Lake Red 6G; Rhodamine Y 20-7425; Rhodamine Zh; Rhodamine 6ZH-DN; Silosuper Pink B; Valley Fast Red 1308

ABSTRACT

Toxicology and carcinogenesis studies of rhodamine 6G were conducted because of potential human exposure related to its use as a dye for natural and synthetic fibers and as a research chemical. These studies were conducted by administering rhodamine 6G (greater than 95% pure) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies (0, 310, 620, 1,250, 2,500, or 5,000 ppm), all five male and five female rats that received 5,000 ppm and 1/5 male rats that received 2,500 ppm died before the end of the studies; all mice lived to the end of the studies. The final mean body weights of rats that received 2,500 ppm were lower than the initial weights. The final mean body weights of mice that received 2,500 or 5,000 ppm were 8% or 18% lower than that of controls for males and 2% or 8% lower for females.

In the 13-week studies, all rats lived to the end of the studies (dietary concentrations of 0 or 120-2,000 ppm). The final mean body weights of rats that received 500, 1,000, or 2,000 ppm were 12%, 13%, or 32% lower than that of controls for males and 4%, 8%, or 20% lower for females. Feed consumption by rats that received 2,000 ppm was somewhat lower than that by controls. Bone marrow atrophy was observed at increased incidences and severity in dosed rats. In the 13-week study (0 or 500-8,000 ppm), 1/10 male mice that received the highest concentration died before the end of the study. The final mean body weights of mice that received 8,000 ppm were lower than the initial mean body weights. The final mean body weights of male mice that received 4,000 ppm and of female mice that received 2,000 or 4,000 ppm were 13%-19% lower than those of controls. Feed consumption was not related to dose. Minimal-to-moderate cytoplasmic vacuolization of hepatocytes was seen in 8/10 male mice that received 8,000 ppm.

Based on these results, dietary concentrations selected for the 2-year studies were 0, 120, or 250 ppm rhodamine 6G for rats, 0, 1,000, or 2,000 ppm for male mice, and 0, 500, or 1,000 ppm for female mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of dosed rats were similar to those of controls throughout the studies. The average daily feed consumption by dosed rats was within 5% that by controls for all dosed groups. The average amount of rhodamine 6G consumed per day was approximately 5 mg/kg for low dose rats and 10 or 12 mg/kg for high dose male or female rats. Mean body weights of high dose male and dosed female mice were generally 5%-14% lower than those of controls. The average daily feed consumption by dosed mice was within 5% that by controls for all dosed groups. The average amount of rhodamine 6G consumed per day was approximately 210 or 440 mg/kg for low dose or high dose male mice and 125 or 250 mg/kg for low dose or high dose female mice. No significant differences in survival were observed between any groups of rats or mice (male rats: control, 22/50; low dose, 21/50; high dose, 27/50; female rats: 29/50; 30/50; 30/50; male mice: 36/50; 32/50; 38/50; female mice: 39/50; 35/50; 36/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: No chemically related nonneoplastic lesions in male or female rats and no chemically related neoplastic or nonneoplastic lesions in male or female mice were observed in these studies.

The incidence of keratoacanthomas of the skin was increased in high dose male rats (control, 1/50; low dose, 2/50; high dose, 8/50). The historical incidence of keratoacanthomas in untreated control male F344/N rats is 31/1,936 (1.6%; range, 0/50-7/49). Both fur and skin of rats in the dosed groups apparently were exposed to feed dust containing rhodamine 6G; the intensity of staining was proportional to the concentration of rhodamine 6G in feed. Because of the variable background incidence of keratoacanthomas in F344/N rats, the incidence of keratoacanthomas cannot be conclusively related to exposure to rhodamine 6G.

The incidences of pheochromocytomas (3/50; 3/50; 8/50) or malignant pheochromocytomas (combined: 3/50; 3/50; 10/50) of the adrenal gland were increased in high dose female rats. The historical incidence of adrenal medullary neoplasms in untreated control F344/N female rats is 99/1,968 (5%; range, 0/50-8/50). This marginal increase may be related to the administration of rhodamine 6G.

Genetic Toxicology: Rhodamine 6G was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with and without exogenous metabolic activation (S9). Rhodamine 6G gave a positive response in the absence of S9 in the mouse lymphoma assay for induction of trifluorothymidine (Tft) resistance in L5178Y cells; in the presence of S9, rhodamine 6G was negative. Rhodamine 6G induced sister chromatid exchanges (SCEs) and chromosomal aberrations in cultured CHO cells in the presence, but not the absence, of S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** for male F344/N rats administered rhodamine 6G, as indicated by a marginally increased incidence of integumentary keratoacanthomas. There was *equivocal evidence of carcinogenic activity* for female F344/N rats administered rhodamine 6G, as indicated by a marginal increase in pheochromocytomas or malignant pheochromocytomas (combined) of the adrenal gland. There was *no evidence of carcinogenic activity* for male B6C3F$_1$ mice administered 1,000 or 2,000 ppm rhodamine 6G in the diet. There was *no evidence of carcinogenic activity* for female B6C3F$_1$ mice administered 500 or 1,000 ppm rhodamine 6G in the diet.

There were no significant nonneoplastic lesions attributed to rhodamine 6G administration to male or female rats or male or female mice. Male and female rats might have been able to tolerate a higher concentration of rhodamine 6G in the feed.

SUMMARY OF THE TWO-YEAR FEED AND GENETIC TOXICOLOGY STUDIES OF RHODAMINE 6G

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Dietary concentrations			
0, 120, or 250 ppm rhodamine 6G	0, 120, or 250 ppm rhodamine 6G	0, 1,000, or 2,000 ppm rhodamine 6G	0, 500, or 1,000 ppm rhodamine 6G
Body weights in the 2-year study			
Dosed groups similar to or higher than controls	Dosed groups similar to or higher than controls	High dose group lower than controls	Dosed groups lower than controls
Survival rates in the 2-year study			
22/50; 21/50; 27/50	29/50; 30/50; 30/50	36/50; 32/50; 38/50	39/50; 35/50; 36/50
Nonneoplastic effects			
None	None	None	None
Neoplastic effects			
Keratoacanthomas of the integumentary system (1/50; 2/50; 8/50)	Pheochromocytomas or malignant pheochromocytomas (combined) of the adrenal gland (3/50; 3/50; 10/50)	None	None
Level of evidence of carcinogenic activity			
Equivocal	Equivocal	No evidence	No evidence
Genetic toxicology			
Salmonella Gene Mutation	Mouse L5178Y/TK Tft Resistance	CHO Cells in Vitro SCE	CHO Cells in Vitro Aberration
Negative with and without S9	Positive without S9; negative with S9	Negative without S9; positive with S9	Negative without S9; positive with S9

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 9.

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 because of major flaws cannot be evaluated ("Inadequate Study"). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports 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 quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either 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 chemically 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 chemically related.

- **No Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing no chemically 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. This 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:

- The 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 incidences 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);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The 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.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of Rhodamine 6G is based on the 13-week studies that began in March 1980 and ended in June 1980 and on the 2-year studies that began in December 1980 and ended in December 1982 at Southern Research Institute (Birmingham, AL).

National Toxicology Program
(Evaluated Experiment, Interpreted Results, and Reported Findings)

John Edgar French, Ph.D., Study Scientist

John R. Bucher, Ph.D.
Scot L. Eustis, D.V.M., Ph.D.
Joseph K. Haseman, Ph.D.
James Huff, Ph.D.

(Discipline Leaders and Principal Contributors)

Jack Bishop, Ph.D.
Douglas W. Bristol, Ph.D.
R. Chhabra, Ph.D.
R. Griesemer, D.V.M., Ph.D.
C.W. Jameson, Ph.D.
E.E. McConnell, D.V.M.
G.N. Rao, D.V.M., Ph.D.
B.A. Schwetz, D.V.M., Ph.D.
Douglas Walters, Ph.D.

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report for Rats on 10/23/85)

Kunitoshi Mitsumori, D.V.M., Ph.D. (Chair) (NTP)
Michael Elwell, D.V.M., Ph.D. (NTP)
Scot L. Eustis, D.V.M., Ph.D. (NTP)
Hershell Giles, D.V.M., Ph.D. (Southern Research Institute)
Peter Millar, M.V.M. (Experimental Pathology Laboratories, Inc.)
Linda Uraih, D.V.M. (NTP)

(Evaluated Slides and Prepared Pathology Report for Mice on 7/16/87)

Robert Sauer, V.M.D. (Chair) (PATHCO, Inc.)
John Cullen,V.M.D., Ph.D. (North Carolina State University)
Scot L. Eustis, D.V.M., Ph.D. (NTP)
Micheal Jokinen, D.V.M. (NTP)
Joel Leininger, D.V.M., Ph.D. (NTP)
Margarita McDonald, D.V.M., Ph.D. (NTP)
Suzanne Neuenschwander, D.V.M. Experimental Pathology Laboratories, Inc.
Roger Thompson, D.V.M., Ph.D. (Southern Research Institute)

Principal Contributors at Southern Research Institute
(Conducted Studies and Evaluated Tissues)

J. Prejean, Ph.D.
H. Giles, D.V.M., Ph.D.
A. Killmeyer, B.S.
R. Thompson, D.V.M., Ph.D.

Principal Contributors at Experimental Pathology Laboratories, Inc.
(Provided Pathology Quality Assurance)

S. Neuenschwander, D.V.M.
Jerry Hardisty, D.V.M.

Principal Contributors at Carltech Associates, Inc.
(Contractor for Technical Report Preparation)

William D. Theriault, Ph.D.
Abigail C. Jacobs, Ph.D.
John Warner, M.S.
Naomi Levy, B.A.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on rhodamine 6G on October 3, 1988, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee

Robert A. Scala, Ph.D.* (Chair)
Senior Scientific Advisor, Medicine and Environmental Health Department
Research and Environmental Health Division, Exxon Corporation
East Millstone, NJ

Michael A. Gallo, Ph.D. (Principal Reviewer)
Associate Professor, Director of Toxicology
Department of Environmental and Community Medicine, UMDNJ - Robert Wood Johnson Medical School, Piscataway, NJ

Frederica Perera, Dr. P.H.
Division of Environmental Sciences
School of Public Health
Columbia University
New York, NY

Ad Hoc Subcommittee Panel of Experts

John Ashby, Ph.D.
Imperial Chemical Industries, PLC
Central Toxicology Laboratory
Alderley Park, England

Robert H. Garman, D.V.M.
Bushy Run Laboratories
Export, PA
Consultants in Veterinary Pathology
Murrysville, PA

Lois Swirsky Gold, Ph.D. (Principal Reviewer)
University of California
Lawrence Berkeley Laboratory
Berkeley, CA

Curtis D. Klaassen, Ph.D.
Professor, Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

William Lijinsky, Ph.D.*
Director, Chemical Carcinogenesis
Frederick Cancer Research Facility
Frederick, MD

Barbara McKnight, Ph.D.
Assistant Professor, Department of Biostatistics, University of Washington
Seattle, WA

Franklin E. Mirer, Ph.D.
Director, Health and Safety Department
International Union, United Auto Workers, Detroit, MI

Paul M. Newberne, D.V.M., Ph.D.
Professor, Mallory Institute of Pathology
Boston, MA

James A. Popp, D.V.M., Ph.D.
Head, Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

*Unable to attend

SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF RHODAMINE 6G

On October 3, 1988, the draft Technical Report on the toxicology and carcinogenesis studies of rhodamine 6G received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.E. French, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (equivocal evidence of carcinogenic activity for male and female rats, no evidence of carcinogenic activity for male and female mice).

Dr. Gold, a principal reviewer, agreed with the conclusions but felt that there was also justification for an evaluation of no evidence of carcinogenic activity for male and female rats. She noted that the historical control incidences were quite variable for keratoacanthomas in male rats and for pheochromocytomas in female rats and that the incidences in high dose groups were similar to the highest spontaneous incidences at the same laboratory in studies conducted during the same time period. Dr. French acknowledged the variability in the historical controls but noted that concurrent controls are most appropriate for comparisons. With regard to the pheochromocytomas, a contributing factor was the observation of malignant tumors in the high dose group. Dr. Gold noted that the International Agency for Research on Cancer had originally evaluated rhodamine 6G as having limited evidence of carcinogenicity on the basis of other (non-NTP) studies. [See page 13.] Dr. Gold inquired about an observation in the Abstract that the fur of control rats was tinged pink. Dr. French responded that this statement was in error and would be deleted from the final Report.

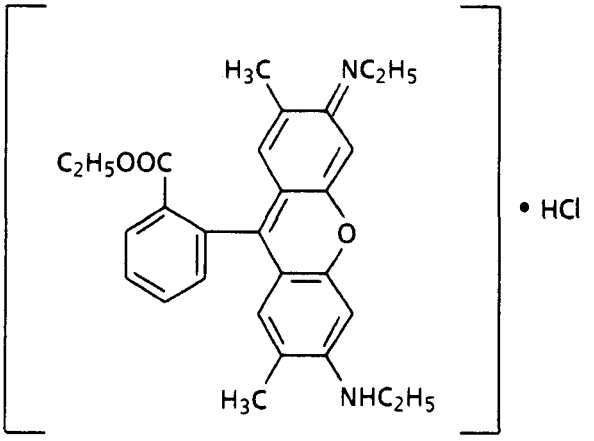
Dr. Gallo, the second principal reviewer, agreed with the conclusions. He speculated that chemical interaction with the epidermal growth factor receptor complex may have played a role in the induction of skin tumors in male rats. Rhodamine compounds are photoactive, and many photoactive compounds have been shown to perturb this receptor complex.

Dr. Gold moved that the conclusions for male and female mice be accepted as written, no evidence of carcinogenic activity. Dr. Gallo seconded the motion, which was approved unanimously by seven members. Dr. Gallo moved that the conclusions for male and female rats be accepted as written, equivocal evidence of carcinogenic activity. Dr. Garman seconded the motion, which was approved by five panelists (Drs. Gallo, Garman, McKnight, Mirer, and Popp) with one dissent (Dr. Gold) and one abstention (Dr. Newberne).

I. INTRODUCTION

Use, Production, and Exposure
Absorption, Metabolism, and Excretion
Reproductive and Developmental Toxicity
Toxicity in Animals
Carcinogenicity
In Vitro Toxicity
Genetic Toxicology
Study Rationale

I. INTRODUCTION



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Use, Production, and Exposure

Rhodamine 6G is used as a dye for silk, cotton, wool, bast fibers, paper, leather, and plastics (Colour Index, 1971; Farris, 1984); a component of C.I. Solvent Red 36; a tracing agent in water pollution studies (Rochat et al., 1975, 1977; Thacker et al., 1984); and an adsorption indicator, especially in very acid solutions (Matsuyama, 1966). As a dye and a fluorescent probe, rhodamine 6G is also used in research on mitochondrial (Bereiter-Hahn et al., 1983; Berns et al., 1984; Dietzmann et al., 1987) and synaptosomal functions (Aiuchi et al., 1982, 1984; Kashiwayanagi et al., 1987), in laser surgery (L'Esperance, 1985a,b), as an insecticide (Pimprikar and Heitz, 1984), in microbiology (Sobczak, 1985), and in drug screening (Halfman and Jay, 1986). Essentially, the compound is used only as a functional dye.

Rhodamine 6G is manufactured by condensing 3-ethylamino-4-methylphenol with phthalic anhydride, followed by esterification with chloroethane under pressure (Cesark, 1970), or with ethanol and a mineral acid (Farris, 1984). Highly concentrated liquid forms have also been prepared by reaction of the rhodamine base with a dialkyl sulfate and a saturated aliphatic glycol at 100°-160° C. Production volume (U.S. import and production) increased from 340,000 kg in 1976 to 1,400,000 kg in 1980. Estimates indicate that approximately 15,000 workers in the paper, chemical and allied products, and

printing and publishing industries may have been exposed to rhodamine 6G (NIOSH, 1974).

Absorption, Metabolism, and Excretion

Rhodamine 6G and rhodamine B were reported to be excreted in the pancreatic juice in situ after intravenous infusion of 1 mg dye per minute to dogs (strain, age, and sex not specified) followed by the administration of secretin or cholecystokinin-pancreozymin stimulation (Hong, 1974). The rate of excretion was not reported.

Reproductive and Developmental Toxicity

Rhodamine 6G was found to be toxic and to cause reproductive embryotoxicity in mice (TD_{Lo}, 4 mg/kg, 7-10 days gestation) (Jones et al., 1986; Ranganathan and Hood, 1986).

Toxicity in Animals

Injections of 0, 5, or 7 mg rhodamine 6G/kg per day into adult male albino mice (35-45 g) for 14 days or two 14-day periods separated by a 21-day break resulted in dose-related decreases in body weight and rectal temperature and increases in adrenal gland, liver, kidney, and spleen weights but not in thymus weight (Soler et al., 1982).

Yoho et al. (1973) found that dietary administration of rhodamine 6G to house flies at concentrations as low as 0.063% in the presence of natural light was 100% lethal. When exposure occurred in the dark, mortality was reduced to 40% of that of the controls. However, Respicio and Heitz (1981) reported that rhodamine 6G in feed was toxic to female common house flies (LC_{50} = 0.67 $\times 10^{-3}$ M) and that toxicity was not dependent on light but was greatest in the dark.

Carcinogenicity

In early studies on rhodamine 6G, Umeda (1956) reported that 40 male and 40 female mice (mixed Saitama strain weighing 20 g) fed a rice diet containing rhodamine 6G at 200 or 500 ppm for 100 days did not develop tumors. There were no controls in this study. In a companion study, Umeda (1956) reported that 7 of 16 rats (200 g, sex not specified, mixed Saitama strain) received 1 ml subcutaneous injections of a 0.02% aqueous solution of rhodamine 6G two to three times per week for 4 months, followed by a series of 100 subcutaneous injections after a 1-month respite. Four of the seven survivors developed fibrosarcomas, two of which were successfully transplanted to other rats of the same strain. No data from concurrent control rats were reported. Based on these studies, rhodamine 6G was initially classified as having limited evidence of carcinogenicity in rats (IARC, 1978), but after re-evaluation, it was moved to a level of insufficient evidence (IARC, 1987).

In Vitro Toxicity

Rhodamine 6G is a potent inhibitor of mitochondrial oxidative phosphorylation (Gear, 1974; Higuti et al., 1980). At low rhodamine concentrations, ATP-dependent calcium ion uptake is blocked (K_i = 3 µM); at concentrations greater than 20 µM, respiration becomes uncoupled and respiration-dependent calcium ion uptake is inhibited (Gear, 1974). Higuti et al. (1980) cited evidence that rhodamine 6G inhibited H^+ ejection from mitochondria energized with ATP or with succinate and postulated that inhibition sites of rhodamine 6G are on membrane components related to H^+ ejection by oxidation/reduction components. Rhodamine 6G has also been found to inhibit the import and processing of matrix-catalyzed mitochondrial proteins in cell-free or cultured human fibroblasts (electron factor flavoprotein) (Ikeda et al., 1986) and in isolated hepatoma ascites cells or normal hepatocytes (e.g., cytochrome b-c_1 complex subunits) (Kolarov and Hatalova, 1984; Kolarov and Nelson, 1984; Kuzela et al., 1986) from male Sprague Dawley rats at concentrations that did not uncouple mitochondrial respiration.

Lampidis et al. (1984) observed that the positively charged dyes rhodamine 6G and rhodamine 123 inhibit heartbeat and kill Sprague Dawley neonatal rat cardiac muscle cells in vitro but the neutral dyes rhodamine B and rhodamine 116 do not. Cationic rhodamine dyes, but not neutral dyes, inhibit oxidative phosphorylation in isolated mitochondria. The investigators also observed differences in the accumulation of rhodamine 123 and rhodamine 6G in cardiac and carcinoma cells. Both dyes selectively inhibit

the in vitro growth (Summerhayes et al., 1982; Lampidis et al., 1985; Wilkie and Fearon, 1985) and in vivo growth (Fearon et al., 1987) of neoplastic cell lines. Lampidis et al. (1985) attributed the selective inhibition and killing of neoplastic cells to the lipophilic positively charged character of these dyes and the difference in transmembrane potential between normal and neoplastic cells. On this basis, positively charged lipophilic dyes such as rhodamine 6G and rhodamine 123 have been proposed as potential antineoplastic agents. Attempts to enhance the selective killing effects of intramitochondrial rhodamine dyes using photolysis were unsuccessful (Oseroff et al., 1986). O'Brian and Weinstein (1987) found that rhodamine 6G inhibits protein kinase C after activation with the tumor promoter 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), presumably through chemical-lipid interaction and the induction of cytotoxicity, but not in the absence of lipid cofactor.

Rhodamine 6G is a specific inhibitor of aerobic growth of yeast (*Saccharomyces cerevisiae*), and isolated rhodamine-6G-resistant mutants have been used to demonstrate extrachromosomal inheritance in yeast (Carignani et al., 1977; Nichols et al., 1977). Ziegler and Davidson (1981) used chloramphenicol-resistant Chinese hamster fibroblast and mouse 3T3-4E cell lines and alternate pretreatment with rhodamine 6G to demonstrate control of mitochondrial determinants in mammalian cell hybrids. The effects of rhodamine 6G on the role of mitochondria in the maternal transmission of an antigen specific for a murine cell-surface molecule (reactive to specific *H-2* nonrestricted cytotoxic T lymphocytes) have also been demonstrated (Smith et al., 1983; Huston et al., 1985). Most inbred strains of mice (with the exception of NZB substrains) are positive for the maternally transmitted antigen. Rhodamine 6G inhibited mitochondrial function and partially restricted or prevented the transmission and expression of the maternally transmitted antigen and demonstrated the role and requirement for functional mitochondria. This phenomenon has also been observed in variants of the human cell line VA_2-B which are resistant to rhodamine 6G and rhodamine 123 (Wiseman et al., 1985).

Genetic Toxicology

Rhodamine 6G did not induce reverse gene mutations when tested with and without S9 metabolic activation in several strains of *Salmonella typhimurium* at a dose of 1.1 µg/plate (Milvy and Kay, 1978) or within a dose range of 0-1,000 µg/plate (Wuebbles and Felton, 1985; Zeiger et al., 1987). A study by Nestmann et al. (1979) reported strong mutagenic activity (up to a thirty-fold increase in revertants over background) in *S. typhimurium* strains TA98, TA100, TA1537, and TA1538 treated with up to 1,000 µg/plate rhodamine 6G in the presence of induced S9. However, a subsequent report from that same laboratory (Matula et al., 1982) attributed the previously observed mutagenic activity of rhodamine 6G to the presence of impurities not detected in the original chemical analysis of the commercial-dye mixture tested. Ultrapurified rhodamine 6G was not mutagenic in Salmonella or *S. cerevisiae*. Nestmann et al. (1979) also reported DNA single-strand breaks, detected by alkaline sucrose sedimentation, and a decrease in colony-forming ability (an indication of impaired survival) in cultured Chinese hamster ovary (CHO) cells exposed to commercial rhodamine 6G at a concentration of about 43 µg/ml for 1 hour in the presence of S9. The possibility that these effects were due to impurities was not resolved. DNA damage did occur at concentrations that induced only slightly impaired survival. Au and Hsu (1979) detected no induction of chromosomal aberrations in CHO cells exposed at 20 µM (9.58 µg/ml) rhodamine 6G in the absence of S9.

The structural analog, rhodamine B, has been tested for mutagenicity in a variety of in vitro and in vivo assays and exhibits a pattern of activity similar to rhodamine 6G. Rhodamine B was negative in tests for induction of DNA damage in *Bacillus subtilis* (Kada et al., 1972; Matsui, 1980), but several observations of gene mutation in Salmonella in the presence of S9 activation have been reported (Brown et al., 1979; Nestmann et al., 1979, 1980; Ishidate et al., 1981). Wuebbles and Felton (1985) and Parodi et al. (1981) observed no increase in Salmonella revertants after exposure to rhodamine B. All

the laboratories reporting Salmonella test results used rhodamine B from different or unidentified sources, and purity was not always specified. Therefore, as with the positive results noted for rhodamine 6G, contaminants in the dye lots tested may be responsible for at least a portion of the observed mutagenic activity. In fact, Nestmann et al. (1979) tested purified rhodamine B and found that the eightfold increase in revertants which they observed in *S. typhimurium* strains TA98 and TA1538 after treatment with commercial rhodamine B in the presence of S9 was reduced to slightly more than a doubling of the background rate. They concluded that most of the mutagenic activity seen in Salmonella after treatment with commercial rhodamine B was attributable to the contaminants present in the mixture. Further support for this belief comes from the studies of Brown et al. (1979), who also compared the mutagenic activity of rhodamine B from different sources and checked the level of impurities in each. They also concluded that the mutagenicity of rhodamine B was due, in large part, to contaminants. Nestmann et al. (1979) reported that, like rhodamine 6G, commercial rhodamine B induced DNA single-strand breaks and decreased survival in CHO cells exposed for 1 hour in the presence of S9; in contrast to the results with rhodamine 6G, toxicity was high relative to the amount of induced DNA damage. Induction of chromosomal aberrations by rhodamine B in the absence of S9 was reported in cultured mammalian cells (Au and Hsu, 1979; Ishidate et al., 1981; Lewis et al., 1981), but results of in vitro cytogenetic tests for induction of sister chromatid exchanges (SCEs) and chromosomal breaks in human fibroblasts were negative (Sasaki et al., 1980). Rhodamine B, administered at a dose of 90 mg/kg by intraperitoneal injection, did not induce SCEs in bone marrow cells of Swiss male mice or DNA strand breaks in male Sprague Dawley rat liver cells in vivo (Parodi et al., 1981, 1983).

Study Rationale

Rhodamine 6G was nominated and selected for study because of its large production volume, potential for worker exposure, and the lack of adequate toxicity and carcinogenicity data. Administration of rhodamine 6G in feed was chosen in order to obtain a systemic exposure.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF RHODAMINE 6G

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES AND FORMULATED DIETS

SINGLE-ADMINISTRATION STUDIES

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

- Study Design
- Source and Specifications of Animals
- Animal Maintenance
- Clinical Examinations and Pathology
- Statistical Methods

GENETIC TOXICOLOGY

PROCUREMENT AND CHARACTERIZATION OF RHODAMINE 6G

Rhodamine 6G--2-[6-(ethylamino)-3-(ethylimino)-2,7-dimethyl-3*H*-xanthen-9-yl] benzoic acid ethyl ester, monohydrochloride--was obtained in one lot (lot no. 14-6907) from BASF Wyandotte Corporation (Parsippany, NJ). Purity and identity determinations were conducted by Midwest Research Institute (MRI) (Kansas City, MO). MRI reports on analyses performed in support of the rhodamine 6G studies are on file at the National Institute of Environmental Health Sciences.

Lot no. 14-6907 was obtained as a red, fluffy microcrystalline powder that sublimated at temperatures from 190° to 280° C. Spectroscopic analysis confirmed the identity of the study material as rhodamine 6G. The infrared (Figure 1) and nuclear magnetic resonance (Figure 2) spectra were consistent with the literature spectra (Sadtler Standard Spectra; Horobin and Murgatroyd, 1969). The ultraviolet/visible spectrum was consistent with that expected for the structure.

The purity of rhodamine 6G was determined by elemental analysis, Karl Fischer water analysis, titration of one amine group, thin-layer chromatography, and high-performance liquid chromatography. Cumulative data indicated that lot no. 14-6907 was greater than 95% pure. The result of elemental analysis for nitrogen agreed with the theoretical value; that for carbon was slightly lower than the theoretical value, whereas those for chlorine and hydrogen were slightly high. Water content was 2.1%. Potentiometric titration of one amine group with 0.1 N perchloric acid in glacial acetic acid containing mercuric acetate indicated a purity of 95.8%.

Thin-layer chromatography on silica gel plates with a diethylamine mobile phase detected four minor impurities and a slight trace impurity with ultraviolet (254 and 366 nm) and visible light visualization. Thin-layer chromatography with a methanol:2-ethoxyethanol:ammonium hydroxide (75:15:5) mobile phase on Whatman KC_{18} reversed-phase plates with fluorescent indicator detected one minor impurity, three trace impurities, and one slight trace impurity by the same visualization methods. Five impurities were detected by high-performance liquid chromatography on a µBondapak C_{18} column (with a mobile phase of aqueous 5 mM heptanesulfonic acid, sodium salt, in water containing 1% acetic acid:5 mM heptanesulfonic acid, sodium salt, in methanol, containing 1% acetic acid [53:47] at a flow rate of 1 ml/minute) with ultraviolet detection at 254 nm. The largest impurity, which was not identified, had an area 1.94% that of the major peak. The combined total area of all impurities totaled 2.6% of the major peak area.

Stability studies performed with the same high-performance liquid chromatographic system with a 41.5:58.5 solvent ratio at a flow rate of 2 ml/minute and hexanophenone as the internal standard indicated that rhodamine 6G was stable in the dark for 2 weeks at temperatures up to 60° C. Further confirmation of the stability of the bulk chemical during the toxicology studies (storage at 22° C) was obtained by ultraviolet spectroscopy at 248 and 348 nm and by high-performance liquid chromatography with the same system but with a 42:58 solvent ratio and a flow rate of 1 ml/minute. Results for the bulk chemical were compared with those for a frozen reference standard. No degradation was seen over the course of the studies. Upon receipt of rhodamine 6G at the study laboratory, the identity was confirmed by infrared spectroscopy.

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES AND FORMULATED DIETS

For the single-administration studies, dose mixtures were prepared by mixing rhodamine 6G with water (Table 1). For the 14-day, 13-week, and 2-year studies, formulated diets were prepared by adding a dry premix to the appropriate amount of feed. The homogeneity of diet mixtures formulated at the analytical chemistry and study laboratories was evaluated by extracting feed samples (taken from three locations in the blender) with methanol:acetic acid (99:1) and determining the absorbance at 528 nm. At the analytical chemistry laboratory, values ranged from 99.0% to 102.3% of the target value at a concentration of 10,000 ppm. At the study laboratory, values ranged from 97.9% to 100.4% of

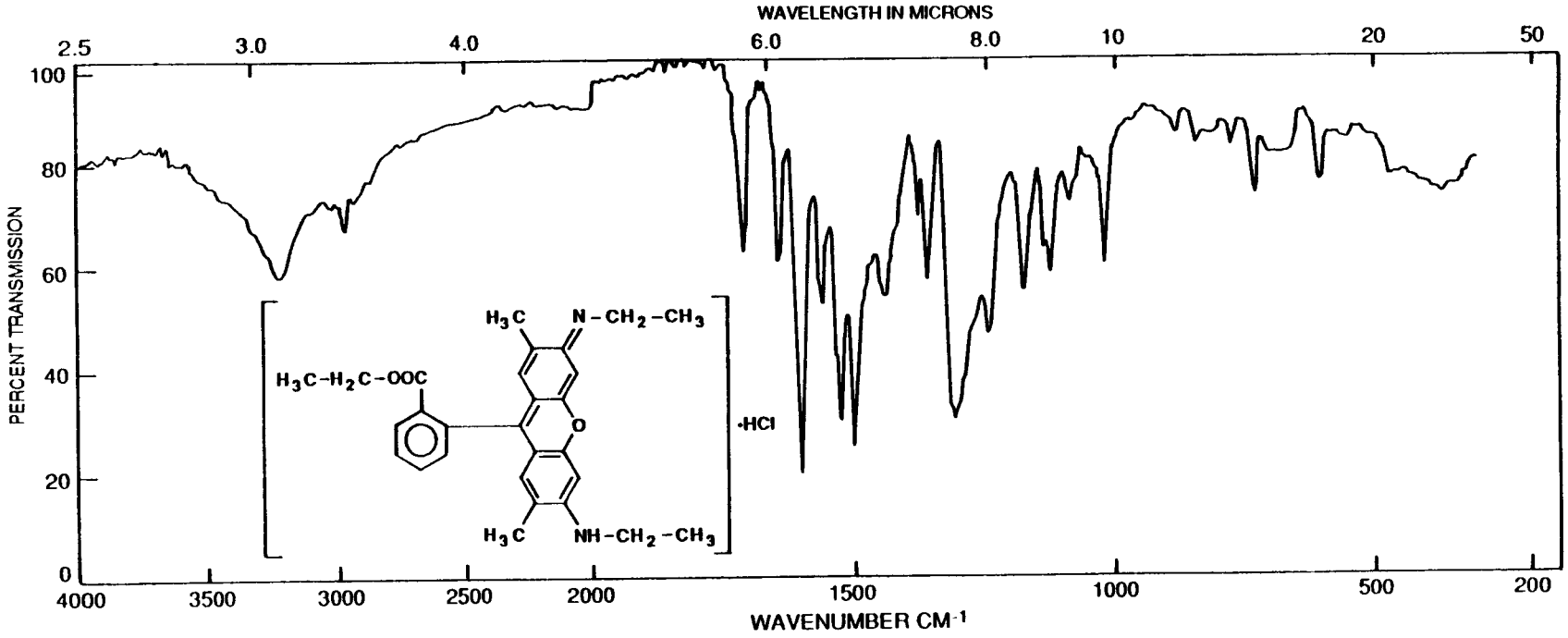

FIGURE 1. INFRARED ABSORPTION SPECTRUM OF RHODAMINE 6G (LOT NO. 14-6907)

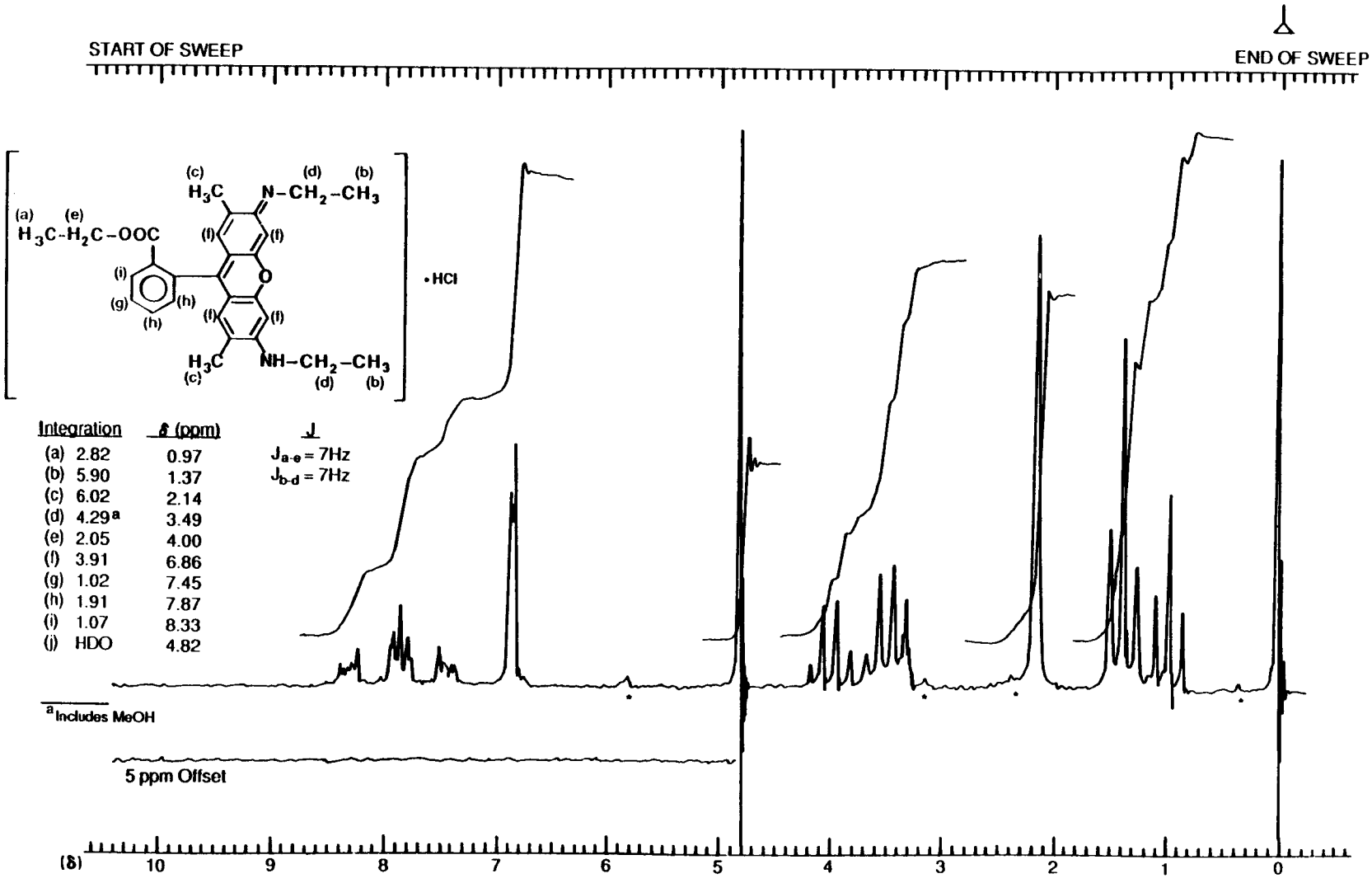

FIGURE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF RHODAMINE 6G (LOT NO. 14-6907)

TABLE 1. PREPARATION AND STORAGE OF DOSE MIXTURES AND FORMULATED DIETS IN THE STUDIES OF RHODAMINE 6G

Single-Administration Studies	Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Preparation			
Rhodamine 6G was mixed with water in serum bottles on magnetic stirrer with stir bar until visually homogenous	Feed was mixed with rhodamine 6G in a specimen cup and shaken for 1 min; premix was mixed with remainder of feed in a 16-qt blender for 15 min	Same as 14-d studies	Same as 14-d studies
Maximum Storage Time			
	2 wk	2 wk	2 wk
Storage Conditions			
	Room temperature	22° C	22° C

the target value at a concentration of 8,000 ppm and from 99.2% to 106.7% at 120 ppm. Further studies indicated that rhodamine 6G was stable in feed (10,000 ppm) when stored for 2 weeks in the dark at temperatures up to 45° C. In these studies, samples were extracted as described above and analyzed by high-performance liquid chromatography on a μBondapak C_{18} column with a mobile phase of aqueous 1% acetic acid: 1% acetic acid in methanol (20:80) at a flow rate of 1 ml/minute; octanophenone was the internal standard, and detection was at 254 nm. Formulated diets were stored at 22° C for no longer than 14 days.

Periodic analysis for rhodamine 6G in formulated diets was performed by the study and analytical chemistry laboratories by the same extraction (100% methanol at the analytical chemistry laboratory) and spectrophotometric quantitation steps used in the homogeneity studies to determine if the formulated diets contained the desired concentrations of rhodamine 6G. Formulated diets were analyzed once during the 13-week studies. The results ranged from 99.3% to 105.0% of the target concentration (Table 2). During the 2-year studies, the formulated diets were analyzed approximately every 8 weeks. The feed mixtures were estimated to have been within ±10% of the target concentration 96% (91/95) of the time (Table 3). All mixtures were within ±15% of the target concentrations. Results of periodic referee analysis performed by the analytical chemistry laboratory indicated generally good agreement with the results from the study laboratory (Table 4).

TABLE 2. RESULTS OF ANALYSES OF FORMULATED DIETS IN THE THIRTEEN-WEEK FEED STUDIES OF RHODAMINE 6G (a)

Target Concentration (ppm)	Determined Concentration (ppm)	Percent of Target
120	(b) 124	103.1
250	257	102.8
500	505	101.0
1,000	1,030	103.0
2,000	2,090	104.5
4,000	4,200	105.0
8,000	(b) 7,947	99.3

(a) Date mixed: 4/9/80; results of duplicate analysis.
(b) Average of values obtained from three locations in the blender

TABLE 3. RESULTS OF ANALYSES OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF RHODAMINE 6G (a)

Date Mixed	Concentration of Rhodamine 6G in Feed for Target Concentration (ppm) (a)				
	120	250	500	1,000	2,000
12/15/80	133	253	503	1,010	2,030
	117	251			
01/12/81	130	259	518		2,120
02/09/81	129	250		1,010	
03/09/81	127	252	522		(b) 2,210
03/12/81					(c) 2,030
04/06/81	127	246		996	
05/04/81	(b) 135	252	492		1,990
05/06/81	(c) 112				
05/28/81	121	243		972	
06/29/81	129	258	514		2,020
07/20/81	122	261		1,020	
08/24/81	112	232	460		1,870
09/21/81	114	251		966	
10/19/81	114	254	484		1,910
11/09/81	121	244		978	
12/07/81	125	249	484		1,880
01/25/82	121	248	510	1,020	2,000
	132	255			
03/22/82	126	248	483	1,019	2,001
	119	253			
05/17/82	114	247	468	949	1,910
	114	245			
07/12/82	115	243	458	944	(b) 1,700
	116	244			
07/14/82					(c) 1,890
09/07/82	120	240	481	944	1,880
	(d) 137	236			
09/10/82		(c) 245	(c) 472	(c) 980	(c) 1,860
		(c) 244			
11/01/82	112	246	464	1,020	1,960
	108	248			
Mean (ppm)	121.9	248.4	488.6	988.31	1,962.9
Standard deviation	7.92	6.72	21.87	30.68	122.21
Coefficient of variation (percent)	6.5	2.7	4.5	3.1	6.2
Range (ppm)	108-137	232-261	458-522	944-1,020	1,700-2,210
Number of samples	27	27	14	13	14

(a) Results of duplicate analysis
(b) Out of specifications; not used in studies.
(c) Remix; not included in the mean.
(d) Out of specifications; used in studies.

TABLE 4. RESULTS OF REFEREE ANALYSES IN THE TWO-YEAR FEED STUDIES OF RHODAMINE 6G

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory (a)	Referee Laboratory (b)
01/12/81	500	518	520
06/29/81	2,000	2,020	1,960
12/07/81	2,000	1,880	2,030
05/17/82	120	114	123
09/07/82	120	119	120

(a) Results of duplicate analysis
(b) Results of triplicate analysis

SINGLE-ADMINISTRATION STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories and observed for 15 days before the studies began. The animals were 7 weeks old when placed on study. Rats were fasted overnight and mice were fasted for 4 hours before they were dosed.

Groups of five rats of each sex were administered a single dose of 31, 62, 125, 250, or 500 mg/kg rhodamine 6G in water by gavage. Groups of five mice of each sex were administered 62, 125, 250, 500, or 1,000 mg/kg. Animals were observed two times per day for 2 weeks. Controls were not used. Details of animal maintenance are presented in Table 5.

FOURTEEN-DAY STUDIES

Four- to five-week-old F344/N rats and 4- to 6-week-old B6C3F$_1$ mice of each sex were obtained from Charles River Breeding Laboratories and were observed for 14 days before being placed on study.

Groups of five males and five females of each species were fed diets containing 0, 310, 620, 1,250, 2,500, or 5,000 ppm rhodamine 6G for 14 consecutive days. Animals were housed five per cage. Feed and water were available ad libitum. Further details of animal maintenance are given in Table 5.

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to rhodamine 6G and to determine the concentrations to be used in the 2-year studies.

Four-week-old F344/N rats and 4- to 6-week-old B6C3F$_1$ mice of each sex were obtained from Charles River Breeding Laboratories. Rats were observed for 21 days and mice for 14 days before being placed on study.

Groups of 10 rats of each sex were fed diets containing 0, 120, 250, 500, 1,000, or 2,000 ppm rhodamine 6G for 13 weeks. Groups of 10 mice of each sex were fed diets containing 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm. Animals were housed five per cage. Feed and water were available ad libitum.

Animals were observed two times per day; moribund animals were killed. Feed consumption was measured once per week by cage. Individual animal weights were recorded once per week. Further experimental details are summarized in Table 5.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 5.

TABLE 5. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF RHODAMINE 6G

Single-Administration Studies	Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN			
Size of Study Groups			
5 males and 5 females of each species	5 males and 5 females of each species	10 males and 10 females of each species	50 males and 50 females of each species
Doses			
Rats--31, 62, 125, 250, or 500 mg/kg rhodamine 6G in water by gavage; dose vol--5 ml/kg; mice--62, 125, 250, 500, or 1,000 mg/kg; dose vol--10 ml/kg	0, 310, 620, 1,250, 2,500, or 5,000 ppm rhodamine 6G in feed	Rats--0, 120, 250, 500, 1,000, or 2,000 ppm rhodamine 6G in feed; mice--0, 500, 1,000, 2,000, 4,000, or 8,000 ppm	Rats--0, 120, or 250 ppm rhodamine 6G in feed; mice--male: 0, 1,000, or 2,000 ppm; female: 0, 500, or 1,000 ppm
Date of First Dose			
9/26/79	1/23/80	3/12/80	Rats--12/25/80; mice--12/18/80
Date of Last Dose			
N/A	2/5/80	6/10/80	Rats--12/19/82; mice--12/8/82
Duration of Dosing			
Single administration	14 consecutive d	13 wk	103 wk
Type and Frequency of Observation			
Observed 2 × d; weighed initially	Observed 2 × d; weighed initially and then 1 × wk; feed consumption measured 1 × d	Observed 2 × d; weighed initially and then 1 × wk; feed consumption measured 1 × wk	Observed 2 × d; weighed initially, 1 × wk for 13 wk, and then 1 × mo
Necropsy and Histologic Examinations			
No necropsy or histologic exams performed	Necropsy performed on all animals; histologic exams not performed	Necropsy performed on all animals; histologic exams performed on all controls, all rats in the 2,000-ppm groups, and all mice in the 8,000-ppm groups. Tissues examined include: adrenal glands, brain, colon, esophagus, femur including marrow, gallbladder (mice), heart, kidneys, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, pancreas, parathyroid glands, pituitary gland, salivary glands, seminal vesicles/prostate/testes or ovaries/uterus, skin, small intestines, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder	Necropsy and histologic exams performed on all animals; the following tissues were examined: adrenal glands, brain, cecum, colon, duodenum, epididymis/prostate/testes or ovaries/uterus, esophagus, femur including marrow, gallbladder (mice), gross lesions and tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular or mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, preputial/clitoral glands (rats only), rectum, salivary glands, skin, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder
ANIMALS AND ANIMAL MAINTENANCE			
Strain and Species			
F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source			
Charles River Breeding Laboratories (Wilmington, MA)	Charles River Breeding Laboratories (Kingston, NY)	Rats--Charles River Breeding Laboratories (Kingston, NY); mice--Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Kingston, NY)

TABLE 5. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF RHODAMINE 6G (Continued)

Single-Administration Studies	Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)			
Study Laboratory			
Southern Research Institute	Southern Research Institute	Southern Research Institute	Southern Research Institute
Method of Animal Identification			
Ear mark	Ear mark	Ear mark	Ear mark
Time Held Before Study			
15 d	14 d	Rats--21 d; mice--14 d	14 d
Age When Placed on Study			
7 wk	Rats--6-7 wk; mice--6-8 wk	Rats--7 wk; mice--6-8 wk	Rats--6-7 wk; mice--7-8 wk
Age When Killed			
9 wk	Rats--8-10 wk; mice--8-11 wk	Rats--20-21 wk; mice--19-23 wk	111-113 wk
Necropsy or Kill Date			
Killed 10/11/79	Rats--2/5/80-2/9/80; mice--2/7/80-2/9/80	Rats--6/12/80-6/21/80; mice--6/13/80-6/23/80	Rats--12/27/82-1/3/83; mice--12/16/82-12/22/82
Method of Animal Distribution			
Animals distributed to weight classes and then assigned to cages by one table of random numbers and to groups by another table of random numbers	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Feed			
Wayne Lab Blox® (Allied Mills, Chicago, IL); available ad libitum	NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as 14-d studies	Same as 14-d studies
Bedding			
Beta chips--heat-treated hardwood chips (Northeastern Products Corp., Warrensburg, NY)	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Water			
Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Cages			
Polycarbonate (Lab Products, Inc., Garfield, NJ)	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Cage Filters			
Reemay spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Animals per Cage			
5	5	5	5
Other Chemicals on Study in the Same Room			
None	None	None	None

TABLE 5. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF RHODAMINE 6G (Continued)

Single-Administration Studies	Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)			
Animal Room Environment			
Temp--69.8°-73.4° F; hum--30%-50%; fluorescent light 12 h/d; at least 15 room air changes/h	Temp--71.6°-73.4° F; hum--34%-43%; fluorescent light 12 h/d; 15 room air changes/h	Temp--71.6°-75.2° F; hum--39%-57%; fluorescent light 12 h/d; at least 15 room air changes/h	Temp--72.9° ± 1.1° F (range: 64°-82° F); hum--51% ± 4% (range: 33%-84%); fluorescent light 12 h/d; more than 15 room air changes/h

TWO-YEAR STUDIES

Study Design

Groups of 50 rats of each sex were fed diets containing 0, 120, or 250 ppm rhodamine 6G for 103 weeks. Groups of 50 male mice received diets containing 0, 1,000, or 2,000 ppm rhodamine 6G and groups of 50 female mice received diets containing 0, 500, or 1,000 ppm.

Source and Specifications of Animals

The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Charles River Breeding Laboratories. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats were shipped to the study laboratory at 4-5 weeks of age and mice at 5-6 weeks of age. The animals were quarantined at the study laboratory for 2 weeks. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. The rats were placed on study at 6-7 weeks of age and the mice at 7-8 weeks of age. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix E).

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid B6C3F$_1$ study animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoresis profiles that demonstrate phenotype expressions of known genetic loci.

The C57BL/6N mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice monitored were variant at one to three loci, indicating some heterogeneity in the C3H line from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks.

Male mice from the C3H colony and female mice from the C57BL/6N colony were used as parents for the hybrid B6C3F$_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because concurrent controls were included in each study.

Animal Maintenance

Animals were housed five per cage. Feed and water were available ad libitum. Cages were not rotated during the studies. Further details of animal maintenance are given in Table 5.

Clinical Examinations and Pathology

All animals were observed two times per day, and clinical signs were recorded at least once per month. Body weights were recorded once per week for the first 13 weeks of the study and once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all animals including those found dead, except for tissues that were excessively autolyzed or missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues examined microscopically are listed in Table 5.

When the pathology evaluation was completed by the laboratory pathologist and the pathology data entered into the Toxicology Data Management System, the slides, paraffin blocks, and residual formalin-fixed tissues were sent to the NTP Archives. The slides, individual animal data records, and pathology tables were sent to an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assessment pathologist. The quality assessment report and slides were submitted to the Pathology Working Group (PWG) Chairperson, who reviewed all target tissues and those about which there was a disagreement between the laboratory and quality assessment pathologists.

Representative slides selected by the Chairperson were reviewed by the PWG, which included the laboratory pathologist, without knowledge of previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the laboratory pathologist was asked to reconsider the original diagnosis. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Slides/tissues are generally not evaluated in a blind fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle or unless there is an inconsistent diagnosis of lesions by the laboratory pathologist. Nonneoplastic lesions are not examined routinely by the quality assessment pathologist or PWG unless they are considered part of the toxic effect of the chemical.

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 were censored from the survival analyses at the time they were found to be missing or dead from other than natural causes; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences were detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared

at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence: The majority of tumors in this study were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was a logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor 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 it did not significantly enhance the fit of the model. The dosed 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 tumors are incidental, this comparison of the time-specific tumor prevalences also provides a comparison of the time-specific tumor incidences (McKnight and Crowley, 1984).

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

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

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984, 1985) are included for those tumors appearing to show compound-related effects.

GENETIC TOXICOLOGY

Salmonella Protocol: Testing was performed as reported by Ames et al. (1975) with modifications listed below and described in greater detail in Haworth et al. (1983) and Mortelmans et al. (1986). The data presented in this report are included in Zeiger et al. (1987). Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). The study chemical 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 before the addition of soft agar supplemented with L-histidine and D-biotin and subsequent plating on minimal glucose agar plates. Incubation was continued for an additional 48 hours.

Each test consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of the study chemical. The high dose was limited by toxicity or solubility but did not exceed 1 mg/plate. All negative assays were repeated, and all positive assays were repeated under the conditions that elicited the positive response.

A positive response was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants which was not dose related, not reproducible, or of insufficient magnitude to support a determination of

mutagenicity. A response was considered negative when no increase in revertant colonies was observed after chemical treatment.

Mouse Lymphoma Protocol: The experimental protocol is presented in detail by Myhr et al. (1985) and follows the basic format of Clive et al. (1979). All study chemicals were supplied as coded aliquots from Radian Corporation (Austin, TX). The highest dose of the study compound was determined by solubility or toxicity and did not exceed 15 µg/ml. Mouse lymphoma L5178Y cells were maintained at 37° C as suspension cultures in Fischer's medium supplemented with 2 mM L-glutamine, 110 µg/ml sodium pyruvate, 0.05% pluronic F68, antibiotics, and heat-inactivated horse serum; normal cycling time was about 10 hours. To reduce the number of spontaneously occurring trifluorothymidine (Tft)-resistant cells, subcultures were exposed once to medium containing thymidine, hypoxanthine, methotrexate, and glycine for 1 day, to thymidine, hypoxanthine, and glycine for 1 day, and to normal medium for 3-5 days. For cloning, horse serum content was increased and Noble agar was added. Freshly prepared S9 metabolic activation factors were obtained from the liver of either Aroclor 1254-induced or noninduced male F344 rats.

All doses within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 ml of medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with the study chemical continued for 4 hours, after which time the medium plus chemical was removed and the cells were resuspended in 20 ml of fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, 3×10^6 cells were plated in medium and soft agar supplemented with Tft for selection of Tft-resistant cells ($TK^{+/+}$), and 600 cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C under 5% carbon dioxide for 10-12 days. All data were evaluated statistically for both trend and peak response. Both responses had to be significant ($P<0.05$) for a chemical to be considered capable of inducing Tft resistance; a single significant response led to an "equivocal" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Myhr et al. (1985). This assay was initially performed without S9; if a clearly positive response was not obtained, the experiment was repeated with induced S9.

Chinese Hamster Ovary Cytogenetics Assays: Testing was performed as reported by Galloway et al. (1985, 1987) and is described briefly below. Chemicals were sent to the laboratories as coded aliquots from Radian Corporation (Austin, TX). Chemicals were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations 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 (BrdU)-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of the study chemical; the high dose was limited by toxicity or solubility but did not exceed 5 mg/ml.

In the SCE test without S9, CHO cells were incubated for 26 hours with the study chemical in McCoy's 5A medium supplemented with 10% fetal bovine serum, L-glutamine (2 mM), and antibiotics. BrdU was added 2 hours after culture initiation. After 26 hours, the medium containing the study chemical was removed and replaced with fresh medium plus BrdU and colcemid, and incubation was continued for 2 more 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 the chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no study chemical; 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.

In the chromosomal aberration test without S9, cells were incubated in McCoy's 5A medium with the study chemical for 8 hours; colcemid was added, and incubation was continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the chromosomal aberration test with S9, cells were treated with the study chemical and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

For the SCE test, if significant chemical-induced cell cycle delay was seen, incubation time was lengthened to ensure a sufficient number of scorable cells. The harvest time for the chromosomal aberration test was based on the cell cycle information obtained in the SCE test; if cell cycle delay was anticipated, the incubation period was extended approximately 5 hours.

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. For the SCE test, 50 second-division metaphase cells were usually scored for frequency of SCEs per cell from each dose; 100 (more recently, 200) first-division metaphase cells were scored at each dose for the chromosomal aberration test. 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).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. 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. Chromosomal aberration data are presented as percentage of cells with aberrations. As with SCEs, both the dose-response curve and individual dose points were statistically analyzed. A statistically significant ($P<0.003$) trend test or a significantly increased dose point ($P<0.05$) was sufficient to indicate a chemical effect.

III. RESULTS

RATS

SINGLE-ADMINISTRATION STUDIES

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs
Survival
Pathology and Statistical Analyses of Results

MICE

SINGLE-ADMINISTRATION STUDIES

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs
Survival
Pathology and Statistical Analyses of Results

GENETIC TOXICOLOGY

SINGLE-ADMINISTRATION STUDIES

All male and female rats that received 500 mg/kg rhodamine 6G by gavage, 3/5 males and 4/5 females that received 250 mg/kg, and 1/5 males that received 125 mg/kg died before the end of the studies (Table 6). Rats that received 250 or 500 mg/kg were inactive. Final weights were not recorded.

FOURTEEN-DAY STUDIES

All male and female rats that received 5,000 ppm rhodamine 6G and 1/5 males that received 2,500 ppm died before the end of the studies (Table 7). The final mean body weights of rats that received 2,500 ppm were lower than the initial weights. Reported feed consumption by males and females that received 5,000 ppm varied erratically from day to day; feed consumption by other groups was similar to that by controls. Compound-related signs in the 2,500- and 5,000-ppm groups of males and females included diarrhea, ruffled fur, decreased activity, and uncoordinated gait.

TABLE 6. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE SINGLE-ADMINISTRATION GAVAGE STUDIES OF RHODAMINE 6G

Dose (mg/kg)	Survival (a)	Initial Mean Body Weight (b)
MALE (c)		
31	5/5	110 ± 3
62	5/5	112 ± 3
125	(d) 4/5	112 ± 4
250	(e) 2/5	112 ± 4
500	(f) 0/5	111 ± 3
FEMALE (g)		
31	5/5	102 ± 2
62	5/5	99 ± 1
125	5/5	104 ± 3
250	(h) 1/5	103 ± 2
500	(i) 0/5	101 ± 2

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) LD_{50} by probit analysis: 201 mg/kg with a 95% confidence interval of 117-347 mg/kg
(d) Day of death: 3
(e) Day of death: all 2
(f) Day of death: 2,2,2,2,4
(g) LD_{50} by Spearman-Karber procedure: 203 mg/kg with a 95% confidence interval of 155-266 mg/kg
(h) Day of death: 1,2,2,2
(i) Day of death: 2,2,2,3,9

TABLE 7. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE FOURTEEN-DAY FEED STUDIES OF RHODAMINE 6G

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 1	Week 2
MALE							
0	5/5	117 ± 3	188 ± 5	+71 ± 3		16	16
310	5/5	112 ± 1	181 ± 3	+69 ± 3	96	15	15
620	5/5	117 ± 2	183 ± 5	+66 ± 3	97	15	15
1,250	5/5	118 ± 4	172 ± 6	+54 ± 4	91	18	18
2,500	(e) 4/5	114 ± 2	110 ± 3	−6 ± 4	59	14	19
5,000	(f) 0/5	120 ± 2	(g)	(g)	(g)	9	26
FEMALE							
0	5/5	98 ± 3	138 ± 4	+40 ± 1		13	13
310	5/5	99 ± 2	136 ± 3	+37 ± 2	99	14	12
620	5/5	93 ± 1	129 ± 3	+36 ± 2	93	13	12
1,250	5/5	95 ± 0	120 ± 2	+25 ± 2	87	18	16
2,500	5/5	96 ± 2	89 ± 3	−7 ± 3	64	14	16
5,000	(h) 0/5	99 ± 2	(g)	(g)	(g)	11	4

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors of the group ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.
(e) Day of death: 14
(f) Day of death: 9,12,13,13,14
(g) No data are reported due to the 100% mortality in this group.
(h) Day of death: 9,9,9,10,11

THIRTEEN-WEEK STUDIES

All rats lived to the end of the studies (Table 8). The final mean body weights of rats that received 500, 1,000, or 2,000 ppm were 12%, 13%, or 32% lower than that of controls for males and 4%, 8%, or 20% lower for females. Feed consumption by the groups that received 2,000 ppm was somewhat lower than that by the controls. Bone marrow atrophy was observed at increased incidences and severity in dosed rats (control, 0/9 males and 1/10 females, minimal severity; 500 ppm, 5/10 males and 4/10 females, minimal severity; 1,000 ppm, 10/10 males and 8/10 females, mild severity; 2,000 ppm, 10/10 males and 9/9 females, moderate severity). Bone marrow atrophy was not observed at 120 or 250 ppm. Feces of dosed animals were pink.

Dose Selection Rationale: Because of bone marrow atrophy, lower weight gain, and lower feed consumption at higher concentrations, dietary concentrations of rhodamine 6G selected for rats for the 2-year studies were 120 and 250 ppm.

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of dosed male rats were generally 3%-9% greater than those of controls from week 77 to the end of the study (Table 9 and Figure 3). Mean body weights of dosed and control female rats were similar throughout the study. The average daily feed consumption by low dose and high dose rats was 103% and 102% that by controls for males and 95% and 101% for females (Tables F1 and F2). The average amount of rhodamine 6G consumed per day was approximately 5 or 10 mg/kg for low dose or high dose male rats and 5 or 12 mg/kg for low dose or high dose female rats. Both fur and skin of dosed animals were red.

TABLE 8. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF RHODAMINE 6G

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 7	Week 13
MALE							
0	10/10	135 ± 2	354 ± 6	+219 ± 4		15	17
120	10/10	141 ± 2	352 ± 4	+211 ± 4	99	16	18
250	10/10	138 ± 2	353 ± 9	+215 ± 8	100	15	18
500	10/10	137 ± 3	313 ± 8	+176 ± 7	88	14	16
1,000	10/10	138 ± 2	308 ± 5	+170 ± 5	87	14	16
2,000	10/10	138 ± 1	241 ± 5	+103 ± 4	68	14	14
FEMALE							
0	10/10	110 ± 2	197 ± 4	+87 ± 3		12	12
120	10/10	118 ± 2	201 ± 2	+83 ± 2	102	12	11
250	10/10	119 ± 1	198 ± 3	+79 ± 2	101	11	11
500	10/10	112 ± 1	190 ± 2	+78 ± 2	96	12	11
1,000	10/10	112 ± 1	181 ± 1	+69 ± 2	92	10	8
2,000	10/10	112 ± 2	157 ± 3	+45 ± 2	80	10	10

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.

TABLE 9. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF RHODAMINE 6G

Weeks on Study	Control		120 ppm			250 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE								
0	126	50	128	102	50	123	98	50
1	159	50	161	101	50	157	99	50
2	197	50	198	101	50	195	99	50
3	226	50	225	100	50	225	100	50
4	252	50	252	100	50	251	100	50
5	272	50	271	100	50	278	102	50
6	290	50	290	100	50	290	100	50
7	303	50	303	100	50	304	100	50
8	315	50	316	100	50	317	101	50
9	326	50	328	101	50	328	101	50
10	336	50	338	101	50	339	101	50
11	344	50	345	100	50	347	101	50
12	351	50	353	101	50	354	101	50
13	355	50	357	101	50	360	101	50
16	374	50	379	101	50	378	101	50
20	385	50	394	102	50	393	102	50
24	394	50	402	102	50	401	102	50
29	422	50	429	102	50	424	100	50
34	431	50	445	103	50	440	102	50
39	446	50	460	103	50	453	102	50
43	452	50	468	104	50	460	102	50
47	456	50	471	103	50	467	102	50
51	465	50	484	104	50	476	102	50
56	472	50	491	104	50	481	102	49
60	476	50	495	104	50	486	102	49
63	481	50	504	105	50	492	102	49
67	482	50	502	104	50	493	102	48
72	482	50	501	104	50	496	103	46
77	456	50	499	109	49	497	109	46
81	468	50	497	106	47	496	106	46
86	450	47	479	106	46	484	108	45
91	464	41	467	101	43	480	103	44
95	445	39	458	103	33	467	105	38
99	429	35	451	105	30	458	107	36
104	435	22	437	100	23	446	103	27
FEMALE								
0	105	50	107	102	50	105	100	50
1	123	50	126	102	50	124	101	50
2	142	50	144	101	50	142	100	50
3	151	50	154	102	50	151	100	50
4	164	50	167	102	50	163	99	50
5	172	50	175	102	50	172	100	50
6	181	50	184	102	50	180	99	50
7	186	50	190	102	50	185	99	50
8	190	50	195	103	50	190	100	50
9	193	50	197	102	50	194	101	50
10	196	50	200	102	50	197	101	50
11	198	50	203	103	50	198	100	50
12	202	50	206	102	50	201	100	50
13	202	50	207	102	50	204	101	50
16	213	50	217	102	50	211	99	50
20	219	50	226	103	50	217	99	50
24	226	50	232	103	50	224	99	50
29	239	50	244	102	50	236	99	50
34	243	50	252	104	50	244	100	50
39	251	50	252	100	50	252	100	50
43	256	50	263	103	50	257	100	50
47	264	50	268	102	50	262	99	50
51	274	50	280	102	50	273	100	50
56	283	50	290	102	50	283	100	49
60	296	49	301	102	50	292	99	47
63	305	49	314	103	50	305	100	47
67	311	49	321	103	50	313	101	47
72	322	49	333	103	50	322	100	47
77	320	47	323	101	50	329	103	46
81	335	47	345	103	50	338	101	45
86	337	47	348	103	47	338	100	44
91	348	43	356	102	46	344	99	40
95	349	39	360	103	41	348	100	36
99	357	36	365	102	39	348	97	36
104	352	30	369	105	33	347	99	33

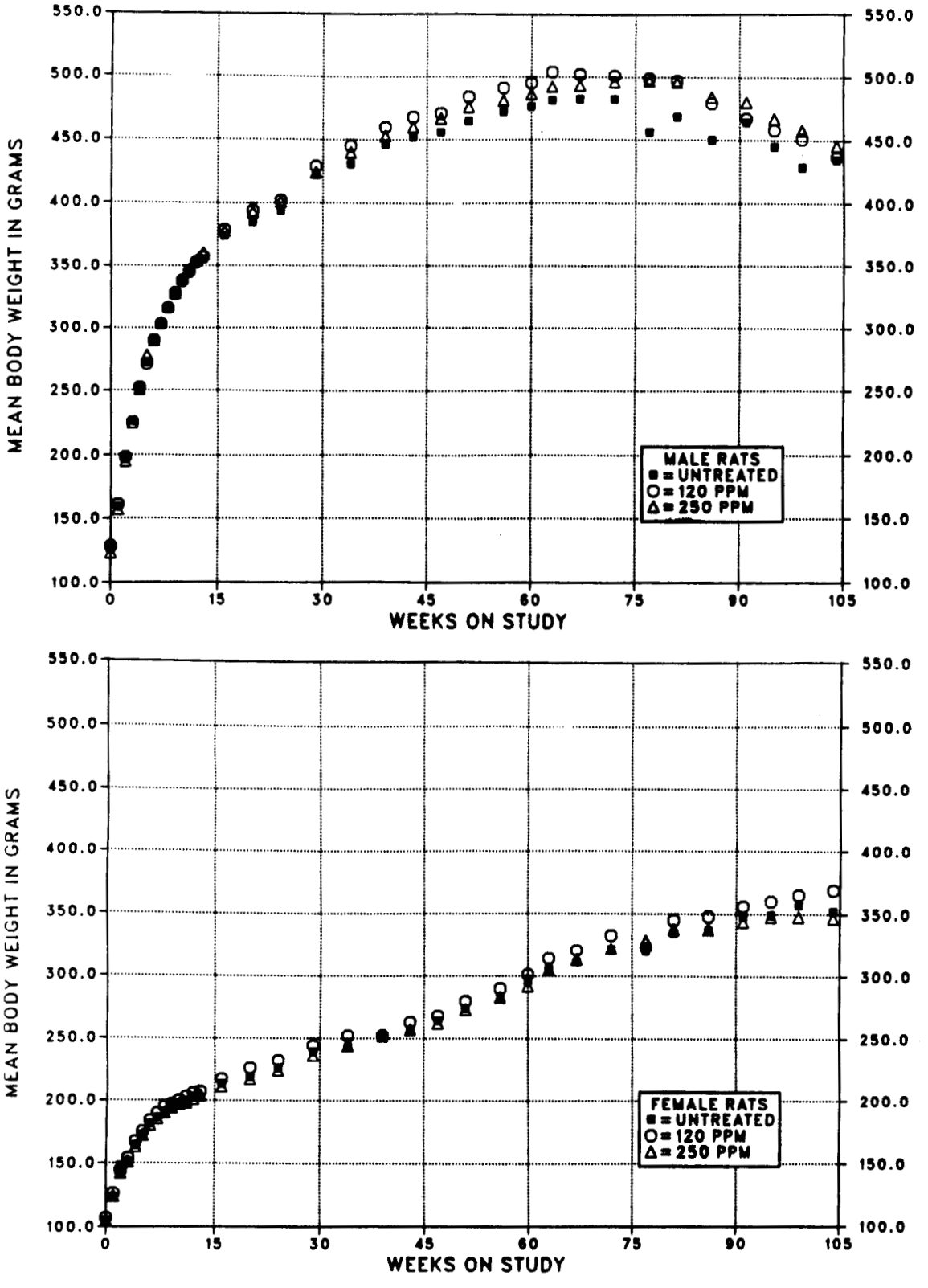

FIGURE 3. GROWTH CURVES FOR RATS FED DIETS CONTAINING RHODAMINE 6G FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female rats fed diets containing rhodamine 6G at the concentrations used in these studies and for controls are shown in Table 10 and in the Kaplan and Meier curves in Figure 4. No significant differences in survival were observed between any groups of either sex.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the skin, adrenal gland, eye, and nose.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group, and historical control incidences for the neoplasms mentioned in this section are presented in Appendixes A and B for male and female rats, respectively.

TABLE 10. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF RHODAMINE 6G

	Control	120 ppm	250 ppm
MALE (a)			
Animals initially in study	50	50	50
Natural deaths	5	5	4
Moribund deaths	23	25	19
Animals surviving until study termination	22	(b) 21	27
Survival P values (c)	0.421	0.809	0.461
FEMALE (a)			
Animals initially in study	50	50	50
Natural deaths	4	4	2
Moribund kills	17	16	18
Animals surviving until study termination	29	30	30
Survival P values (c)	0.990	0.853	1.000

(a) First day of termination period: male--733; female--734
(b) One animal was died or was killed in a moribund condition and was combined, for statistical purposes, with those killed at termination.
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.

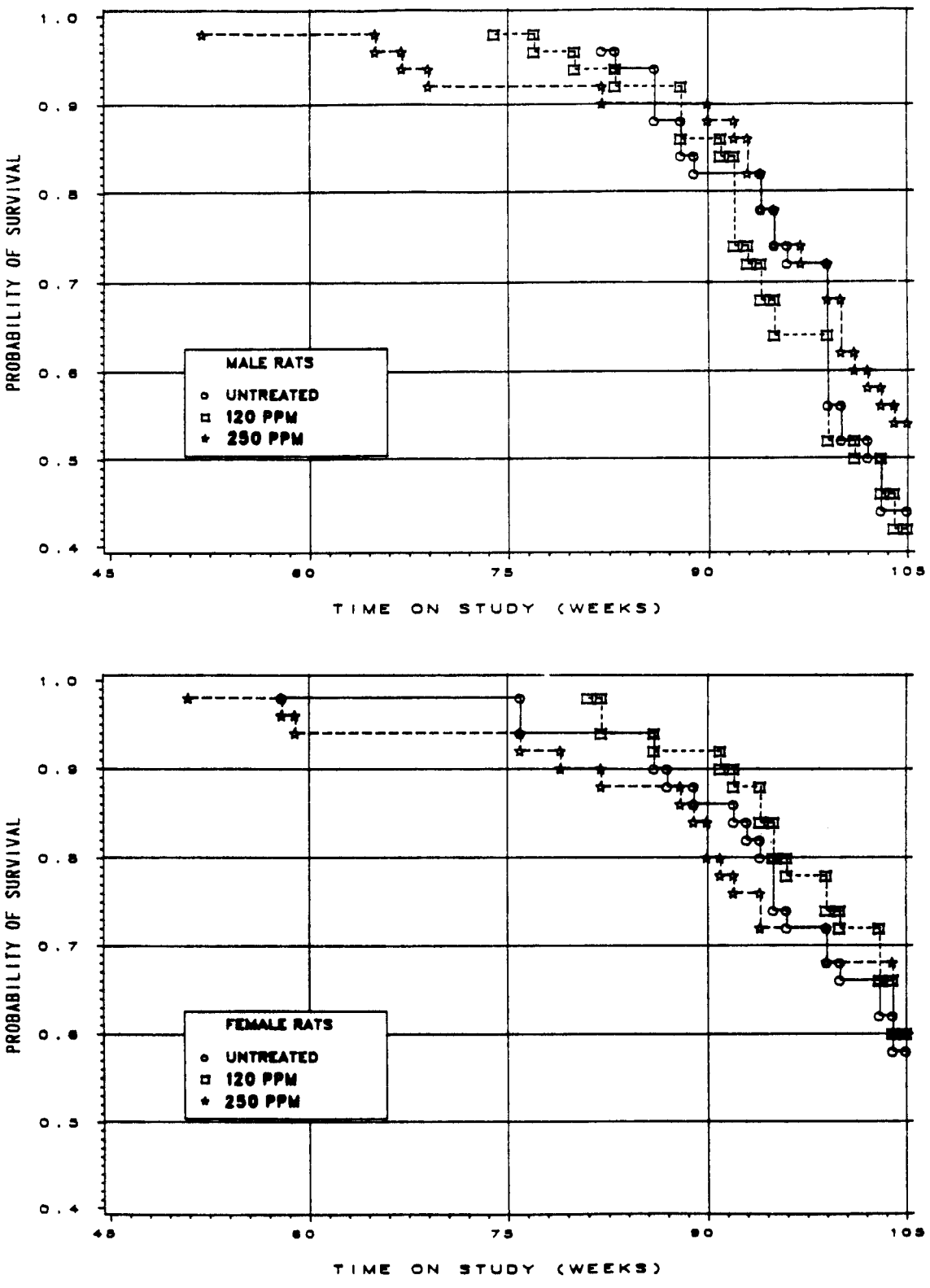

FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING RHODAMINE 6G FOR TWO YEARS

Skin: Keratoacanthomas in male rats occurred with a significant positive trend; the incidence in the high dose group was significantly greater than that in the controls (Table 11). The incidences of keratoacanthomas in female rats were control, 1/50; low dose, 1/50; high dose, 0/50. Keratoacanthoma is an epithelial tumor that may be derived from the hair follicle. The tumor is invaginated beneath the epidermis to form a crater-shaped structure with a central cavity. The wall of the tumor consists of stratified squamous epithelium that forms papillary projections into the center of the cavity. These are typically covered by a thick layer of keratin. The squamous epithelium is well-differentiated without cellular atypia or dysplasia.

Adrenal Gland: Pheochromocytomas or malignant pheochromocytomas (combined) in female rats occurred with a significant positive trend; the incidence in the high dose group was significantly greater than that in the controls (Table 12). Focal hyperplasia of the adrenal medulla was also marginally increased in dosed female rats. Adrenal medullary hyperplasia and pheochromocytomas are part of a morphologic continuum. Pheochromocytomas are distinguished from hyperplasia on the basis of the degree of cellular atypia, extent of alteration in cellular organization or growth pattern, and compression of adjacent tissue. Pheochromocytomas that have extreme cellular anaplasia and/or invade the capsule are considered malignant.

Eye: Retinal degeneration and cataracts were observed at increased incidences in high dose male and low dose female rats (retinal degeneration--male: control, 1/4; low dose, 6/8; high dose, 17/18; female: 2/2; 21/21; 5/6; cataracts--male: 1/4; 3/8; 13/18; female: 1/2; 21/21; 3/6) (denominators are numbers of animals examined microscopically; all animals were examined grossly). Cages were not rotated during the studies; dose columns were rotated throughout the studies (top, high dose; mid, low dose; bottom, control).

Nose: Fungus was observed in 22%-40% of the male rats in each of the groups.

TABLE 11. KERATOACANTHOMAS OF THE SKIN IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (a,b)

	Control	120 ppm (c)	250 ppm (c)
Overall Rates	1/50 (2%)	2/50 (4%)	8/50 (16%)
Terminal Rates	0/22 (0%)	1/21 (5%)	4/27 (15%)
Day of First Observation	667	662	667
Logistic Regression Tests	P=0.006	P=0.503	P=0.018

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table A3 (footnotes).
(b) Historical incidence at study laboratory (mean ± SD): 12/439 (3% ± 5%); historical incidence in NTP studies: 31/1,936 (2% ± 3%)
(c) The estimated dose in milligrams per kilograms per day is given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix F.

TABLE 12. ADRENAL MEDULLARY LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Control	120 ppm	250 ppm
Focal Hyperplasia			
Overall Rates	4/50 (8%)	6/50 (12%)	8/50 (16%)
Pheochromocytoma			
Overall Rates	3/50 (6%)	3/50 (6%)	8/50 (16%)
Terminal Rates	3/29 (10%)	1/30 (3%)	4/30 (13%)
Day of First Observation	734	638	531
Logistic Regression Tests	P=0.053	P=0.644N	P=0.092
Malignant Pheochromocytoma			
Overall Rates	0/50 (0%)	0/50 (0%)	2/50 (4%)
Pheochromocytoma or Malignant Pheochromocytoma (a)			
Overall Rates	3/50 (6%)	3/50 (6%)	10/50 (20%)
Terminal Rates	3/29 (10%)	1/30 (3%)	6/30 (20%)
Day of First Observation	734	638	531
Logistic Regression Tests	P=0.014	P=0.644N	P=0.032

(a) Historical incidence at study laboratory (mean ± SD): 26/436 (6% ± 5%); historical incidence in NTP studies: 99/1,968 (5% ± 4%)

SINGLE-ADMINISTRATION STUDIES

Twenty-nine of 50 mice that received rhodamine 6G by gavage died within 4 days (Table 13). Some animals in all dosed groups were inactive.

FOURTEEN-DAY STUDIES

All mice lived to the end of the studies (Table 14). Mice that received 5,000 ppm gained little or no weight. The final mean body weights of mice that received 2,500 or 5,000 ppm were 8% or 18% lower than that of controls for males and 2% or 8% lower for females. Feed consumption by dosed mice was similar to that by controls. No compound-related clinical signs were observed.

TABLE 13. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE SINGLE-ADMINISTRATION GAVAGE STUDIES OF RHODAMINE 6G

Dose (mg/kg)	Survival (a)	Initial Mean Body Weight (b)
MALE (c)		
62	5/5	27.6 ± 0.9
125	(d) 1/5	30.0 ± 0.6
250	(e) 1/5	30.0 ± 0.5
500	(f) 2/5	29.2 ± 1.1
1,000	(g) 0/5	29.4 ± 1.1
FEMALE (h)		
62	5/5	19.8 ± 0.2
125	(i) 4/5	21.0 ± 0.4
250	(f) 2/5	20.8 ± 0.4
500	(g) 1/5	20.6 ± 0.5
1,000	(j) 0/5	20.2 ± 0.6

(a) Number surviving/number initially in group; LD_{50} values by probit analysis.
(b) Initial group mean body weight ± standard error of the mean
(c) LD_{50} = 145 mg/kg with a 95% confidence interval of 29-304 mg/kg
(d) Day of death: 1,2,2,3
(e) Day of death: 1,1,2,2
(f) Day of death: 1,1,2
(g) Day of death: all 1
(h) LD_{50} = 235 mg/kg with a 95% confidence interval of 131-416 mg/kg
(i) Day of death: 1
(j) Day of death: 1,1,1,3,4

TABLE 14. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE FOURTEEN-DAY FEED STUDIES OF RHODAMINE 6G

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 1	Week 2
MALE							
0	5/5	23.4 ± 0.4	27.8 ± 0.5	+4.4 ± 0.2		6	9
310	5/5	22.4 ± 0.7	27.4 ± 0.7	+5.0 ± 0.4	98.6	6	7
620	5/5	22.8 ± 0.5	28.0 ± 1.0	+5.2 ± 0.6	100.7	7	7
1,250	5/5	23.0 ± 0.4	27.2 ± 0.7	+4.2 ± 1.0	97.8	7	8
2,500	5/5	22.2 ± 0.7	25.6 ± 1.1	+3.4 ± 0.5	92.1	7	8
5,000	5/5	23.2 ± 0.6	22.8 ± 0.4	−0.4 ± 0.2	82.0	7	8
FEMALE							
0	5/5	17.6 ± 0.4	20.2 ± 0.5	+2.6 ± 0.4		7	8
310	5/5	16.8 ± 0.2	19.6 ± 0.5	+2.8 ± 0.5	97.0	6	6
620	5/5	17.4 ± 0.2	20.0 ± 0.4	+2.6 ± 0.2	99.0	7	6
1,250	5/5	17.8 ± 0.4	20.2 ± 0.6	+2.4 ± 0.2	100.0	7	7
2,500	5/5	17.8 ± 0.4	19.8 ± 0.5	+2.0 ± 0.3	98.0	8	7
5,000	5/5	18.4 ± 0.4	18.6 ± 0.4	+0.2 ± 0.4	92.1	7	7

(a) Number surviving/number initially in the group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.

THIRTEEN-WEEK STUDIES

One of 10 male mice that received 8,000 ppm died before the end of the studies (Table 15). The final mean body weights of mice that received 8,000 ppm were lower than the initial mean body weights. The final mean body weights of male mice that received 4,000 ppm and female mice that received 2,000 or 4,000 ppm were notably lower than those of controls. Feed consumption was not related to dose. Minimal-to-moderate cytoplasmic vacuolization of hepatocytes was seen in 8/10 male mice that received 8,000 ppm.

Dose Selection Rationale: Because of lower weight gain at higher concentrations, dietary concentrations of rhodamine 6G selected for mice for the 2-year studies were 1,000 and 2,000 ppm for males and 500 and 1,000 ppm for females.

TWO-YEAR STUDIES

Body Weights, Feed Consumption, and Clinical Signs

Mean body weights of high dose male mice were 5%-14% lower than those of controls from week 6 to the end of the studies (Table 16 and Figure 5). Mean body weights of low dose male mice were generally within 6% of those of controls. Mean body weights of high dose female mice were 6%-11% lower than those of controls from week 29 to week 61 and 10%-14% lower thereafter. Mean body weights of low dose female mice were 6%-13% lower than those of controls after week 35. The average daily feed consumption by low dose and high dose male mice was 99% and 95% that by controls and by low dose and high dose female mice, 99% that by controls (Tables F3 and F4). The average amount of rhodamine 6G consumed per day was approximately 210 or 440 mg/kg for low dose or high dose male mice and 125 or 250 mg/kg for low dose or high dose female mice.

TABLE 15. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF RHODAMINE 6G

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 7	Week 13
MALE							
0	10/10	25.9 ± 0.3	34.4 ± 0.5	+8.5 ± 0.6		8	8
500	10/10	25.0 ± 0.7	34.0 ± 0.9	+9.0 ± 0.6	98.8	8	6
1,000	10/10	25.1 ± 0.6	34.3 ± 1.3	+9.2 ± 0.8	99.7	8	7
2,000	10/10	25.8 ± 0.7	33.5 ± 0.8	+7.7 ± 0.3	97.4	8	6
4,000	10/10	25.4 ± 0.6	27.9 ± 0.5	+2.5 ± 0.6	81.1	7	6
8,000	(e) 9/10	24.5 ± 0.5	19.3 ± 0.4	−5.2 ± 0.4	56.1	8	8
FEMALE							
0	10/10	18.9 ± 0.3	27.0 ± 0.6	+8.1 ± 0.6		8	6
500	10/10	19.0 ± 0.3	26.7 ± 0.6	+7.7 ± 0.5	98.9	8	8
1,000	10/10	18.5 ± 0.4	25.5 ± 0.6	+7.0 ± 0.5	94.4	7	8
2,000	10/10	19.1 ± 0.3	23.6 ± 0.3	+4.5 ± 0.3	87.4	7	7
4,000	10/10	19.2 ± 0.5	22.1 ± 0.4	+2.9 ± 0.3	81.9	7	7
8,000	10/10	18.8 ± 0.4	17.6 ± 0.5	−1.2 ± 0.3	65.2	7	7

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors of the group ± standard error of the mean
(d) Grams per animal per day; not corrected for scatter.
(e) Week of death: 2

TABLE 16. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF RHODAMINE 6G

Weeks on Study	Control Av. Wt. (grams)	Control No. of Survivors	Low Dose Av. Wt. (grams)	Low Dose Wt. (percent of controls)	Low Dose No. of Survivors	High Dose Av. Wt. (grams)	High Dose Wt. (percent of controls)	High Dose No. of Survivors
MALE				**1,000 ppm**			**2,000 ppm**	
0	22.4	50	22.9	102	50	22.7	101	50
1	24.9	50	25.1	101	50	24.5	98	50
2	26.8	50	26.3	98	50	26.1	97	50
3	28.3	50	27.7	98	50	26.8	95	50
4	28.9	50	28.8	100	50	28.2	98	50
5	30.8	50	29.8	97	50	29.5	96	50
6	31.8	50	30.8	97	50	29.3	92	50
7	32.3	50	31.3	97	50	29.9	93	50
8	32.9	50	32.1	98	50	31.0	94	50
9	33.5	50	31.8	95	50	31.0	93	50
10	33.6	50	33.1	99	50	31.8	95	50
11	34.6	50	33.2	96	50	31.9	92	50
12	34.5	50	33.3	97	50	32.4	94	50
13	35.2	49	33.7	96	50	32.6	93	50
17	36.5	49	34.6	95	50	33.4	92	50
21	37.0	49	34.9	94	50	33.8	91	50
25	37.7	49	36.4	97	50	34.2	91	50
29	38.7	49	36.9	95	50	34.9	90	50
35	39.8	49	37.8	95	50	35.3	89	50
40	40.0	49	38.4	96	50	35.7	89	50
43	39.9	49	38.1	95	50	35.4	89	50
48	40.7	49	39.0	96	50	35.9	88	50
52	41.0	49	39.2	96	50	35.6	87	50
57	40.4	49	38.7	96	50	35.4	88	46
61	40.9	49	39.0	95	50	35.4	87	46
64	40.8	49	38.5	94	49	35.9	88	44
68	41.2	48	38.5	93	49	35.8	87	44
73	41.1	48	38.5	94	46	35.5	86	44
78	40.1	48	38.6	96	45	35.8	89	43
82	40.6	47	38.0	94	44	35.2	87	43
87	38.8	46	36.9	95	44	34.5	89	43
91	39.3	44	37.7	96	41	34.7	88	43
95	38.4	40	35.9	93	37	34.4	90	43
99	38.5	39	36.7	95	36	34.2	89	43
104	37.2	36	36.3	98	32	34.7	93	38
FEMALE				**500 ppm**			**1,000 ppm**	
0	17.0	50	17.8	105	50	17.1	101	50
1	18.2	50	17.1	94	50	18.4	101	50
2	19.5	50	19.2	98	50	19.7	101	50
3	20.3	50	20.1	99	50	20.6	101	50
4	20.8	50	20.3	98	50	21.0	101	50
5	22.0	50	21.7	99	50	22.6	103	50
6	22.9	50	22.4	98	50	22.7	99	50
7	23.5	50	22.6	96	50	22.9	97	50
8	23.7	50	22.9	97	50	23.5	99	50
9	24.5	50	23.4	96	50	23.5	96	50
10	24.3	50	22.7	93	50	23.8	98	50
11	24.8	50	24.3	98	50	24.3	98	50
12	24.8	50	24.9	100	50	24.6	99	50
13	25.2	50	24.2	96	50	24.6	98	50
17	26.4	50	25.7	97	50	25.5	97	50
21	27.9	50	26.2	94	50	26.6	95	50
25	28.0	50	27.4	98	50	27.1	97	50
29	30.1	50	28.8	96	50	28.3	94	50
35	31.0	50	29.2	94	50	28.8	93	50
40	32.3	50	29.1	90	50	30.4	94	50
43	32.9	50	29.8	91	50	30.6	93	50
48	34.2	50	31.0	91	50	31.3	92	50
52	34.5	50	32.3	94	50	32.5	94	50
57	35.2	50	32.8	93	50	32.6	93	50
61	36.9	50	32.8	89	50	32.7	89	50
64	37.6	50	33.6	89	49	33.9	90	50
68	37.7	50	33.8	90	49	33.4	89	49
73	39.8	50	35.5	89	49	34.1	86	49
78	39.9	50	34.6	87	49	34.3	86	49
82	38.4	49	34.8	91	48	33.7	88	49
87	37.0	48	34.1	92	46	33.4	90	47
91	38.2	47	35.0	92	46	34.4	90	45
95	38.2	44	34.7	91	44	34.5	90	43
99	39.5	42	35.3	89	39	35.2	89	42
104	40.5	39	35.3	87	35	36.1	89	37

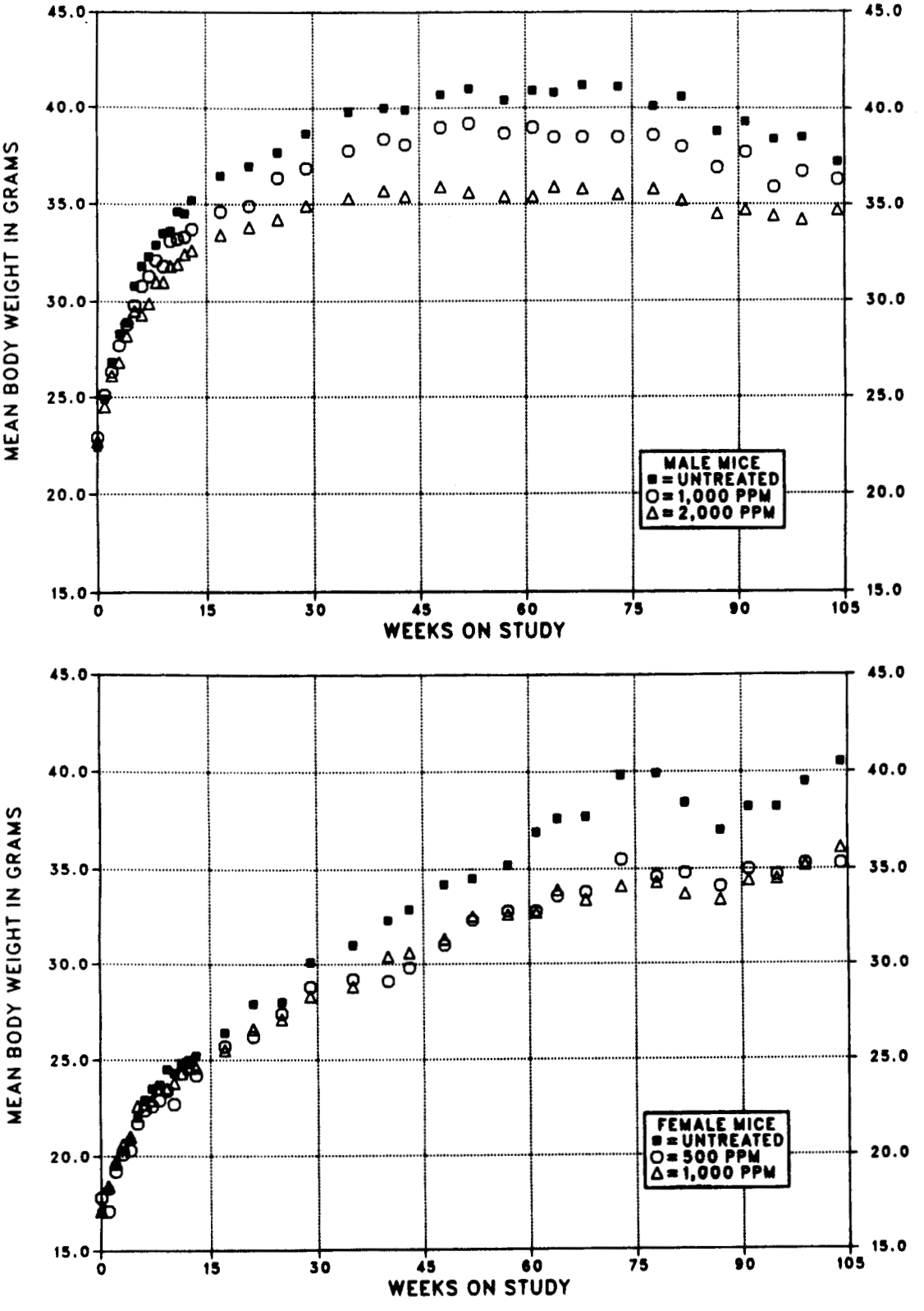

FIGURE 5. GROWTH CURVES FOR MICE FED DIETS CONTAINING RHODAMINE 6G FOR TWO YEARS

Survival

Estimates of the probabilities of survival of male and female mice fed diets containing rhodamine 6G at the concentrations used in these studies and those of controls are shown in Table 17 and in the Kaplan and Meier curves in Figure 6. No significant differences in survival were observed between any groups of either sex.

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the thyroid gland, Harderian gland, brain, and hematopoietic system.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group, and historical control incidences for the neoplasms mentioned in this section are presented in Appendixes C and D for male and female mice, respectively.

TABLE 17. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF RHODAMINE 6G

	Control	500 ppm	1,000 ppm	2,000 ppm
MALE (a)				
Animals initially in study	50		50	50
Natural deaths	5		9	8
Moribund kills	9		8	4
Accidentally killed	0		1	0
Animals surviving until study termination	36		32	38
Survival P values (b)	0.804		0.588	0.863
FEMALE (a)				
Animals initially in study	50	50	50	
Natural deaths	8	7	7	
Moribund kills	3	8	7	
Animals surviving until study termination	39	35	36	
Survival P values (b)	0.569	0.467	0.630	

(a) First day of termination period: 729
(b) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.

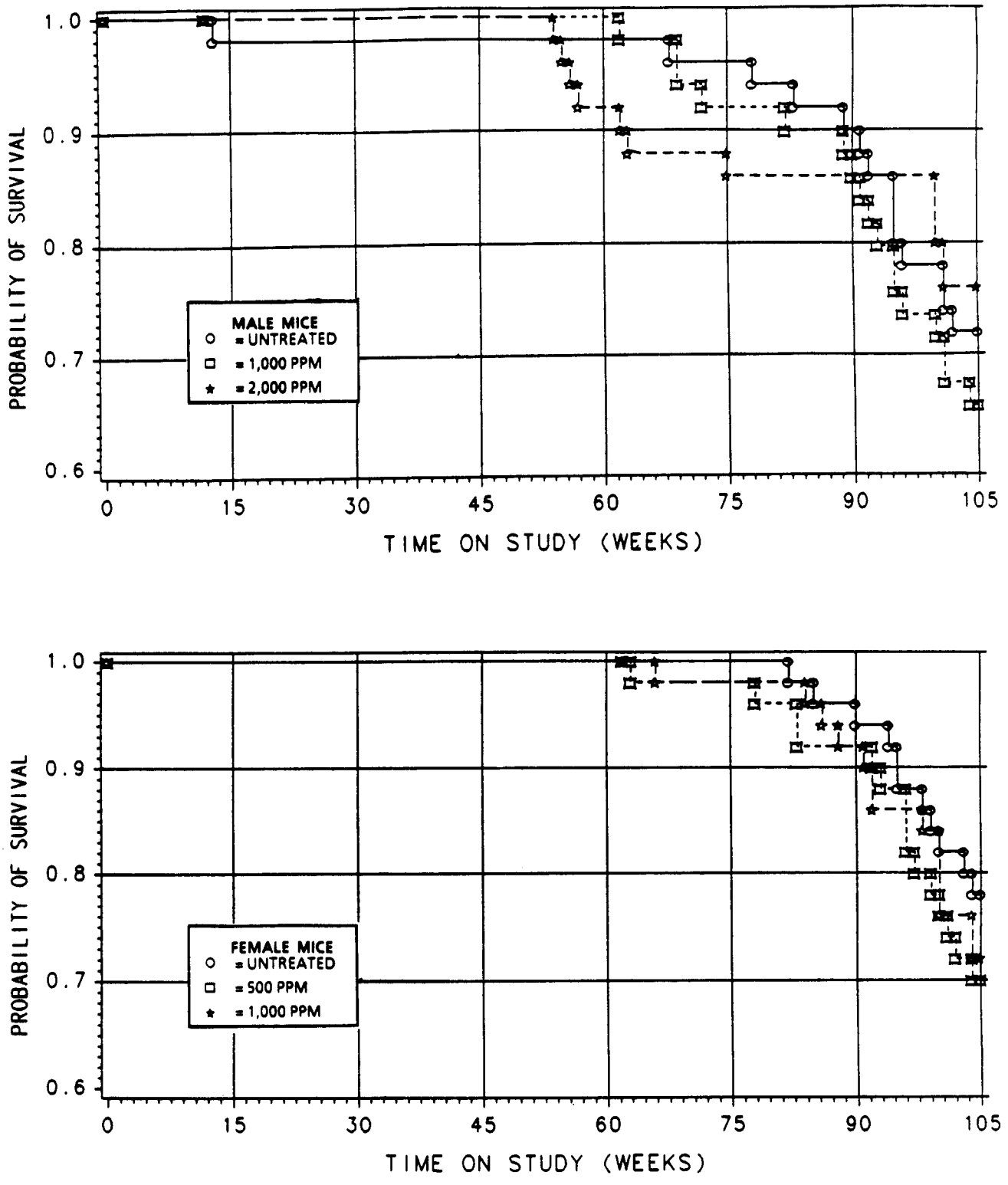

FIGURE 6. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING RHODAMINE 6G FOR TWO YEARS

Thyroid Gland: The incidence of follicular cell adenomas or carcinomas (combined) in low dose male mice was marginally greater than that in controls (Table 18) but was not believed to be related to rhodamine 6G exposure. No dose-response relationship was observed for either follicular cell focal hyperplasia or tumors, and the incidence of follicular cell neoplasms in the high dose group was within the historical incidence at the laboratory (Table C4).

Harderian Gland: Five adenomas or carcinomas (combined) were observed in low dose female mice (Table 19). The Harderian glands were examined microscopically only when there was gross evidence of enlargement of the gland; one control, five low dose, and no high dose female mice were examined microscopically. The incidences in dosed male mice were not increased (control, 7/50; low dose, 2/50; high dose, 2/50). The incidence of Harderian gland neoplasms in low dose female mice is not believed to be related to rhodamine 6G exposure. No dose-response relationship was observed for the neoplasms, and there were no neoplasms in the high dose group.

Brain: Corpora amylacea was observed at increased incidences in dosed male mice (male: control, 2/50; low dose, 12/49; high dose, 10/49; female: 8/50; 6/48; 13/49).

Hematopoietic System: Malignant lymphomas in female mice occurred with a significant negative trend; the incidences in the dosed groups were significantly lower than that in the controls by logistic regression analysis (Table 20).

TABLE 18. THYROID GLAND FOLLICULAR CELL LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (a)

	Control	1,000 ppm (b)	2,000 ppm (b)
Focal Hyperplasia			
Overall Rates	3/50 (6%)	4/49 (8%)	1/50 (2%)
Adenoma			
Overall Rates	0/50 (0%)	3/49 (6%)	3/50 (6%)
Carcinoma			
Overall Rates	0/50 (0%)	1/49 (2%)	0/50 (0%)
Adenoma or Carcinoma (c)			
Overall Rates	0/50 (0%)	4/49 (8%)	3/50 (6%)
Terminal Rates	0/36 (0%)	4/32 (13%)	3/38 (8%)
Day of First Observation		729	729
Logistic Regression Tests	P=0.135	P=0.049	P=0.131

(a) The statistical analyses used are discussed in Section II (Statistical Methods) and Table C3 (footnotes).
(b) The estimated dose in milligrams per kilograms per day is given in Section III (Body Weights, Feed Consumption, and Clinical Signs) and in Appendix F.
(c) Historical incidence at study laboratory (mean ± SD): 11/434 (3% ± 2%); historical incidence in NTP studies: 29/1,958 (1% ± 2%)

TABLE 19. HARDERIAN GLAND LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Control	500 ppm	1,000 ppm
Hyperplasia (a)			
Overall Rates	1/1 (100%)	0/5 (0%)	0/0
Adenoma			
Overall Rates	0/50 (0%)	4/50 (8%)	0/50 (0%)
Carcinoma			
Overall Rates	0/50 (0%)	1/50 (2%)	0/50 (0%)
Adenoma or Carcinoma (b)			
Overall Rates	0/50 (0%)	5/50 (10%)	0/50 (0%)
Terminal Rates	0/39 (0%)	4/35 (11%)	0/36 (0%)
Day of First Observation		709	
Logistic Regression Tests	P=0.591	P=0.027	(c)

(a) Denominators represent animals examined microscopically.
(b) Historical incidence at study laboratory (mean ± SD): 10/448 (2% ± 2%); historical incidence in NTP studies: 48/2,040 (2% ± 2%)
(c) No P value is reported because no tumors were observed in the 1,000-ppm and control groups.

TABLE 20. MALIGNANT LYMPHOMAS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (a)

	Control	500 ppm	1,000 ppm
Overall Rates	16/50 (32%)	8/50 (16%)	7/50 (14%)
Terminal Rates	9/39 (23%)	5/35 (14%)	3/36 (8%)
Day of First Observation	630	440	459
Life Table Tests	P=0.037N	P=0.100N	P=0.056N
Logistic Regression Tests	P=0.012N	P=0.038N	P=0.018N

(a) Historical incidence of lymphomas or leukemia (combined) at study laboratory (mean ± SD): 104/448 (23% ± 7%); historical incidence in NTP studies: 636/2,040 (31% ± 13%)

Rhodamine 6G (97.4% pure) was not mutagenic in any of four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) when tested according to a preincubation protocol at doses up to 1,000 µg/plate in the presence or absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Table 21). When tested at doses up to 10 µg/ml in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y/TK cells, rhodamine 6G gave a positive response in the absence of activation and a negative response with Aroclor 1254-induced male F344 rat liver S9 (Table 22). Rhodamine 6G induced both sister chromatid exchanges (SCEs) and chromosomal aberrations in cultured Chinese hamster ovary cells when tested in the presence of Aroclor 1254-induced male Sprague Dawley rat liver S9; results of both tests were negative in the absence of S9 (Tables 23 and 24). Although some cell cycle delay was noted at higher doses in the SCE test, significant increases in SCE frequencies were observed in cultures harvested at both normal and extended culture times (see trial 2, +S9). Significant increases in chromosomal aberrations were observed only in cells that were allowed additional culture time to offset the rhodamine 6G-induced cell cycle delay.

TABLE 21. MUTAGENICITY OF RHODAMINE 6G IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/Plate (b) −S9 Trial 1	−S9 Trial 2	+S9 (hamster) Trial 1	+S9 (hamster) Trial 2	+S9 (rat) Trial 1	+S9 (rat) Trial 2
TA100	0	98 ± 12.1	131 ± 13.2	137 ± 3.7	181 ± 4.6	121 ± 9.4	160 ± 5.7
	0.3	93 ± 4.0	131 ± 11.9	--	--	--	--
	1	95 ± 5.3	155 ± 2.4	--	--	--	--
	3.3	99 ± 1.7	135 ± 7.0	--	142 ± 5.2	--	--
	6.7	--	--	--	--	--	190 ± 8.4
	10	101 ± 10.3	113 ± 5.8	132 ± 8.6	129 ± 9.3	100 ± 5.7	199 ± 10.4
	16.7	--	--	--	--	--	192 ± 12.8
	33	100 ± 3.8	118 ± 16.1	148 ± 16.4	144 ± 4.4	118 ± 5.3	173 ± 8.8
	67	--	--	--	--	--	155 ± 20.7
	100	--	--	136 ± 4.7	153 ± 8.0	114 ± 3.8	--
	333	--	--	162 ± 6.8	123 ± 8.1	128 ± 2.6	--
	1,000	--	--	71 ± 2.6	--	37 ± 8.4	--
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		1,000 ± 11.7	998 ± 45.5	1,985 ± 74.8	1,169 ± 53.6	467 ± 48.5	1,266 ± 20.0
TA1535	0	9 ± 0.3	12 ± 2.7	10 ± 0.3	15 ± 1.0	14 ± 1.2	16 ± 1.5
	0.3	10 ± 1.8	13 ± 0.7	--	--	--	--
	1	5 ± 1.2	18 ± 4.4	--	--	--	--
	3.3	6 ± 1.0	13 ± 3.1	--	17 ± 1.8	--	15 ± 1.0
	10	8 ± 1.2	16 ± 3.0	14 ± 2.8	15 ± 1.9	12 ± 1.9	21 ± 2.6
	33	7 ± 0.6	14 ± 1.2	17 ± 0.6	14 ± 0.9	15 ± 2.2	20 ± 2.5
	100	--	--	15 ± 1.7	15 ± 2.0	11 ± 1.2	17 ± 0.6
	333	--	--	13 ± 1.2	16 ± 0.9	7 ± 0.9	13 ± 0.9
	1,000	--	--	Toxic	--	Toxic	--
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		346 ± 15.9	892 ± 35.4	158 ± 7.4	372 ± 10.2	215 ± 2.3	345 ± 31.7
TA1537	0	7 ± 2.4	6 ± 1.0	10 ± 1.7	11 ± 2.5	10 ± 0.9	9 ± 1.5
	0.3	10 ± 3.2	6 ± 2.6	--	--	--	--
	1	9 ± 0.6	8 ± 0.7	--	--	--	--
	3.3	13 ± 1.2	8 ± 0.7	--	11 ± 0.9	--	11 ± 1.9
	10	9 ± 1.5	5 ± 0.7	15 ± 1.0	14 ± 0.9	9 ± 1.2	8 ± 0.3
	33	7 ± 0.3	6 ± 1.0	10 ± 2.5	12 ± 3.8	9 ± 0.3	8 ± 1.9
	100	--	--	9 ± 1.3	10 ± 0.7	10 ± 0.9	9 ± 2.3
	333	--	--	10 ± 0.3	8 ± 1.5	9 ± 2.0	8 ± 2.0
	1,000	--	--	0 ± 0.0	--	Toxic	--
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		354 ± 7.4	228 ± 53.1	149 ± 13.7	185 ± 12.9	228 ± 25.8	154 ± 11.5
TA98	0	13 ± 3.2	20 ± 2.3	18 ± 1.9	13 ± 1.2	20 ± 0.3	36 ± 2.6
	0.3	11 ± 0.9	12 ± 2.0	--	--	--	--
	1	14 ± 0.6	14 ± 2.7	--	--	--	--
	3.3	13 ± 0.6	13 ± 1.2	--	13 ± 1.0	--	34 ± 3.7
	10	16 ± 0.9	18 ± 2.3	21 ± 1.0	14 ± 2.1	19 ± 2.9	33 ± 2.7
	33	19 ± 3.8	14 ± 1.2	16 ± 2.7	10 ± 0.3	17 ± 0.3	27 ± 5.9
	100	--	--	20 ± 2.7	8 ± 0.6	25 ± 1.5	25 ± 2.5
	333	--	--	18 ± 0.9	9 ± 0.3	20 ± 1.8	26 ± 2.0
	1,000	--	--	Toxic	--	Toxic	--
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control (c)		366 ± 5.8	157 ± 14.3	1,352 ± 23.4	1,251 ± 32.6	1,662 ± 129.3	1,075 ± 122.4

TABLE 21. MUTAGENICITY OF RHODAMINE 6G IN *SALMONELLA TYPHIMURIUM* (Continued)

(a) Study performed at Case Western Reserve University. The detailed protocol is presented in Haworth et al. (1983) and Mortelmans et al. (1986); the data in this report are included in Zeiger et al. (1987). Cells and study compound or solvent (95% ethanol) were incubated in the absence of exogenous metabolic activation (−S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 µg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA1537.

TABLE 22. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE IN MOUSE L5178Y LYMPHOMA CELLS BY RHODAMINE 6G (a,b)

Compound	Concentration (μg/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Tft-Resistant Cells	Mutant Fraction (c)
-S9					
Trial 1					
Ethanol (d)		91.8 ± 3.8	100.0 ± 5.8	99.8 ± 15.4	36.0 ± 5.7
Rhodamine 6G	(e) 1.25	61.0 ± 9.0	10.5 ± 1.5	88.0 ± 20.0	50.5 ± 18.5
	2.5	58.7 ± 4.9	12.7 ± 0.3	112.0 ± 17.2	(f) 64.3 ± 10.0
	3.75	50.3 ± 4.1	8.3 ± 0.3	61.7 ± 8.2	41.3 ± 6.4
	5	51.7 ± 6.2	7.3 ± 1.9	107.3 ± 12.9	(f) 72.0 ± 13.1
	7.5	Lethal	--	--	--
Methyl methanesulfonate	(e) 5	83.0 ± 9.0	76.5 ± 6.5	584.0 ± 79.0	(f) 233.0 ± 6.0
Trial 2					
Ethanol (e)		56.5 ± 4.5	100.0 ± 6.0	96.0 ± 11.0	56.5 ± 1.5
Rhodamine 6G	2	55.7 ± 4.8	28.3 ± 3.5	79.0 ± 0.6	47.7 ± 3.5
	3	49.0 ± 2.1	20.7 ± 0.7	115.3 ± 10.8	78.3 ± 8.6
	4	50.0 ± 2.1	16.3 ± 1.3	134.3 ± 10.7	(f) 89.7 ± 8.4
	5	57.7 ± 5.3	15.3 ± 2.7	137.7 ± 35.1	78.7 ± 15.8
	(g) 6	49.0 ± 12.0	6.5 ± 1.5	182.5 ± 24.5	(f) 128.5 ± 15.5
	8	Lethal	--	--	--
Methyl methanesulfonate	(e) 5	29.0 ± 3.0	28.0 ± 4.0	345.5 ± 5.5	(f) 402.5 ± 50.5
Trial 3					
Ethanol (d)		81.8 ± 3.9	100.3 ± 13.7	99.3 ± 5.9	40.3 ± 2.0
Rhodamine 6G	2	61.7 ± 3.2	38.0 ± 3.5	71.0 ± 5.1	38.3 ± 2.2
	3	50.7 ± 5.5	32.0 ± 6.0	86.0 ± 9.2	57.0 ± 2.0
	4	51.7 ± 4.7	24.7 ± 4.1	72.7 ± 10.1	48.0 ± 8.5
	5	51.3 ± 1.2	12.7 ± 1.2	121.3 ± 14.9	(f) 78.3 ± 8.5
	6	49.3 ± 0.9	9.3 ± 1.2	137.3 ± 46.8	(f) 92.7 ± 32.2
	8	45.0 ± 7.5	9.0 ± 1.2	166.3 ± 26.3	(f) 134.0 ± 35.5
	10	Lethal	--	--	--
Methyl methanesulfonate	5	69.3 ± 4.1	55.0 ± 3.5	533.7 ± 6.0	(f) 259.7 ± 13.1
+S9 (h)					
Ethanol (d)		86.0 ± 6.7	100.0 ± 6.9	172.8 ± 36.8	65.5 ± 8.5
Rhodamine 6G	2.5	80.7 ± 9.5	48.7 ± 6.5	98.3 ± 8.6	41.3 ± 1.9
	5	70.3 ± 4.3	30.7 ± 3.5	105.3 ± 9.2	50.0 ± 1.7
	7.5	78.3 ± 1.5	23.7 ± 3.0	123.7 ± 6.4	52.7 ± 2.4
	10	56.7 ± 1.2	17.0 ± 0.6	85.0 ± 5.5	50.0 ± 2.6
	15	Lethal	--	--	--
Methylcholanthrene	2.5	67.7 ± 13.1	24.7 ± 5.2	853.3 ± 86.9	(f) 440.3 ± 60.5

TABLE 22. INDUCTION OF TRIFLUOROTHYMIDINE RESISTANCE IN MOUSE L5178Y LYMPHOMA CELLS BY RHODAMINE 6G (Continued)

(a) Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail by Myhr et al. (1985) and follows the basic format of Clive et al. (1979). The highest dose of study compound is determined by solubility or toxicity and may not exceed 5 mg/ml. All doses are tested in triplicate; the average for the three tests is presented in the table. Cells (6×10^5/ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (Tft) for selection of Tft-resistant cells, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.
(b) Mean ± standard error from replicate trials of approximately 1×10^6 cells each. All data are evaluated statistically for both trend and peak response ($P<0.05$ for at least one of the three highest dose sets). Both responses must be significantly ($P<0.05$) positive for a chemical to be considered capable of inducing Tft resistance. If only one of these responses is significant, the call is "equivocal"; the absence of both trend and peak response results in a "negative" call.
(c) Mutant fraction (frequency) is a ratio of the Tft-resistant cells to the cloning efficiency, divided by 3 (to arrive at MF per 1×10^6 cells treated); MF = mutant fraction.
(d) Data presented are the results of four tests.
(e) Data presented are the results of two tests.
(f) Significant positive response; occurs when the relative mutant fraction (average MF of treated culture/average MF of solvent control) is greater than or equal to 1.6.
(g) Data presented are for two tests; the dose in one test was lethal.
(h) Tests conducted with metabolic activation were performed as described in (a) except that S9, prepared from the liver of Aroclor 1254-induced F344 rats, was added at the same time as the study chemical and/or solvent (ethanol).

TABLE 23. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY RHODAMINE 6G (a)

Compound	Dose (μg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hours in BrdU	Relative SCEs/cell (percent) (b)
-S9 (c) Summary: Negative								
Ethanol		50	1,036	386	0.37	7.7	26.5	
Rhodamine 6G	0.0396	50	1,037	362	0.35	7.2	26.5	93.5
	0.132	50	1,034	307	0.30	6.1	26.5	79.2
	0.396	50	1,035	336	0.32	6.7	26.5	87.0
	0.396	50	1,041	335	0.32	6.7	(d) 31.0	87.0
	1.32	0					(d) 31.0	
Mitomycin C	0.0015	50	1,036	512	0.49	10.2	26.5	132.5
	0.01	10	208	208	1.00	20.8	26.5	270.1
+S9 (e)								
Trial 1--Summary: Equivocal								
Ethanol		50	1,046	486	0.46	9.7	26.0	
Rhodamine 6G	1.32	50	1,046	466	0.45	9.3	26.0	95.9
	3.96	50	1,044	499	0.48	10.0	26.0	103.1
	13.2	50	1,047	580	0.55	11.6	26.0	119.6
	39.6	0					(d) 30.0	
Cyclophosphamide	0.4	50	1,048	583	0.56	11.7	26.0	120.6
	2.5	10	210	253	1.20	25.3	26.0	260.8
Trial 2--Summary: Positive								
Ethanol		50	1,035	483	0.47	9.7	26.0	
Rhodamine 6G	9.95	50	1,039	635	0.61	12.7	26.0	130.9
	15	50	1,046	576	0.55	11.5	26.0	118.6
	19.9	50	1,038	722	0.70	14.4	(d) 30.0	148.5
	24.9	0					26.0	
Cyclophosphamide	0.5	50	1,043	618	0.59	12.4	26.0	127.8
	2.5	10	210	308	1.47	30.8	26.0	317.5

(a) Study performed at Bioassay Systems Corporation. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (ethanol) as described in (c) and (e) below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air dried, and stained.
(b) SCEs/cell of culture exposed to study chemical relative to those of culture exposed to solvent
(c) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent for 2 hours at 37° C. Then BrdU was added, and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 hours.
(d) Because some chemicals induce a delay in the cell division cycle, harvest times are occasionally extended to maximize the proportion of second division cells available for analysis.
(e) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with colcemid present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

TABLE 24. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY RHODAMINE 6G (a)

Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
−S9 (b) Harvest time: 10.5 hours				
Ethanol				
	200	5	0.03	2.5
Rhodamine 6G				
0.196	200	6	0.03	3
0.59	200	3	0.02	1.5
1.96	120	1	0.01	0.8
5.9	50	5	0.10	8.0
19.6	0			
Summary: Negative				
Mitomycin C				
1	200	34	0.17	15
5	50	28	0.56	36
+S9 (c) **Trial 1**--Harvest time: 12 hours				
Ethanol				
	200	5	0.03	2.5
Rhodamine 6G				
1.96	200	3	0.02	1.5
5.9	200	3	0.02	1.5
19.6	200	5	0.03	2.5
39.2	0			
Summary: Negative				
Cyclophosphamide				
50	50	32	0.64	24
Trial 3--Harvest time: 20.5 hours (d)				
Ethanol				
	200	2	0.01	1
Rhodamine 6G				
10	200	8	0.04	4
15	200	16	0.08	6
20	200	43	0.22	7.5
25	0			
Summary: Positive				
Cyclophosphamide				
50	10	84	8.40	100
Trial 2--Harvest time: 20 hours (d)				
Ethanol				
	200	16	0.08	7.5
Rhodamine 6G				
9.9	200	17	0.09	8
14.9	200	45	0.23	13.5
19.9	200	42	0.21	9.5
29.9	0			
Summary: Negative				
Cyclophosphamide				
50.0	10	89	8.90	100
Trial 4--Harvest time: 20 hours (d)				
Ethanol				
	200	4	0.02	2
Rhodamine 6G				
9.9	200	5	0.03	2.5
19.8	200	91	0.46	17
29.7	200	168	0.84	28
Summary: Positive				
Cyclophosphamide				
10	50	59	1.18	42
50	10	100	10	100

TABLE 24. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY RHODAMINE 6G (Continued)

(a) Study performed at Bioassay Systems Corporation. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al. (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (100% ethanol) as indicated in (b) and (c). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.
(b) In the absence of S9, cells were incubated with study compound or solvent for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest.
(c) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.
(d) Because of significant chemical-induced cell cycle delay, incubation time prior to addition of colcemid was lengthened to provide sufficient metaphases at harvest.

IV. DISCUSSION AND CONCLUSIONS

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Toxicology and carcinogenesis studies of rhodamine 6G were conducted because of potential human exposure resulting from its use as a dye for natural and synthetic fibers and in biomedical research and because of the absence of information on rhodamine 6G toxicity and potential carcinogenicity. Rhodamine 6G is toxic to eukaryotic cell mitochondria and, depending on cellular concentration, may block ATP-dependent calcium uptake or uncouple mitochondrial respiration (Gear, 1974), inhibit proton movement across the intramitochondrial membrane (Higuti et al., 1980), and inhibit the import and processing of matrix-catalyzed mitochondrial proteins (Kolarov and Hatalova, 1984; Kolarov and Nelson, 1984; Ikeda et al., 1986; Kuzela et al., 1986).

Rhodamine has been shown to be genotoxic in cultured mammalian cells. It induced chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells in the presence of S9 metabolic activation. It also increased the incidence of trifluorothymidine-resistant mouse lymphoma cells in the absence, but not the presence, of S9. Rhodamine 6G was negative in Salmonella tests conducted by the NTP. Previous reports of mutagenic activity with rhodamine 6G in Salmonella have been confirmed by Matula et al. (1982) and are attributed in large part to impurities in the commercial dyes tested.

The toxicity of rhodamine 6G after a single administration was similar in magnitude for F344/N rats and B6C3F$_1$ mice and provided the basis for dose selection in the 14-day studies (up to 5,000 ppm rhodamine 6G). In rats, dietary concentrations of 2,500 ppm or more were apparently not palatable and resulted in no weight gain during the 14-day studies (see Table 7) and a number of deaths. Mice exposed at the highest dose did not gain weight (see Table 14), even though the estimated feed consumption by dosed mice was similar to that by control mice.

In 13-week studies in rats, the maximum dietary concentration of rhodamine 6G was 2,000 ppm. There were no deaths, but there was a reduction in body weight gain relative to controls in male rats given 500 ppm or more and in female rats given 1,000 or 2,000 ppm (see Table 8). The maximum dietary concentration for mice was 8,000 ppm. At 4,000 ppm rhodamine 6G or more, male mice had reduced weight gain or lost weight, whereas females had lower weight gain at 2,000 ppm or more (see Table 15). Other than dose-related reduced weight gain in both rats and mice and a single death in the highest dose group of male mice, the only compound-related effects in the short-term studies were the increased incidences and severity of bone marrow atrophy in male and female rats and cytoplasmic vacuolization of hepatocytes in the highest dose group of male mice. Based on these results, dietary concentrations of 0, 120, or 250 ppm rhodamine 6G were selected for rats for the 2-year studies. Male mice received diets containing 0, 1,000, or 2,000 ppm rhodamine 6G; female mice received diets containing 0, 500, or 1,000 ppm rhodamine 6G because their body weight was less than 90% that of controls at 2,000 ppm.

Mean body weights (see Table 9 and Figure 3) and feed consumption (Tables F1 and F2) of dosed rats were similar to those of controls throughout the 2-year studies, and there were no significant differences in survival (see Table 10 and Figure 4). Mean body weights were reduced 5%-14% relative to controls for dosed mice (see Table 16 and Figure 5), although feed consumption by dosed and control mice was similar (Tables F3 and F4). There were no significant differences in survival in male or female mice (see Table 17). The average amount of rhodamine 6G consumed per day was approximately 5 mg/kg for low dose rats and 10 or 12 mg/kg for high dose male or female rats. The estimated amounts of rhodamine 6G consumed by dosed rats were considerably less than the average estimated amounts of rhodamine 6G consumed per day by low dose or high dose male (210 or 440 mg/kg) or female (125 or 250 mg/kg) mice.

No significant nonneoplastic lesions were associated with chemical exposure in male or female rats or male or female mice in these 2-year studies. Only the increased incidences of keratoacanthomas of the skin in high dose male rats (see Table 11) and pheochromocytomas or malignant pheochromocytomas in high dose female rats (see Table 12) and the reduced body weights (greater than 10%) in dosed male and female mice suggest that rhodamine 6G at the dietary concentrations used in these studies resulted in

biologic effects. The increased incidences of eye lesions (see page 39) are most likely due to cage placement under fluorescent light (top, high dose; mid, low dose; bottom, control) and lack of cage rotation, although photoactivation of rhodamine 6G after systemic exposure cannot be ruled out.

The origin and biologic behavior of keratoacanthomas are not well understood (Turosov, 1979), and the possible induction of this tumor by chemicals or irradiation is not well documented. Squamous cell papillomas, squamous cell carcinomas, and basal cell tumors are the most common chemically induced skin tumors of rats. In the current studies, it is conceivable that the increased incidence of keratoacanthomas of the skin could have resulted from systemic exposure or via direct contact with the skin. Exposure of the skin and fur of dosed male and female rats to rhodamine 6G was evident from the staining of the fur and bedding as a consequence of contact with rhodamine 6G in the ground meal diet and the dust generated. In addition to the evidence of direct skin contact, another factor that affects interpretation of the increase in keratoacanthomas in high dose male rats is the genotoxicity of rhodamine 6G.

Rhodamine 6G is genotoxic. In a survey of 222 chemicals (Ashby and Tennant, 1988) evaluated by the National Cancer Institute (NCI)/NTP for carcinogenicity in rats and mice, six chemicals were identified as inducing skin neoplasms in male rats. All six were genotoxic. Five of these six chemicals are *N*-substituted aromatic compounds, as is rhodamine 6G. Each of these five chemicals induced mutations in Salmonella; however, rhodamine 6G did not. Benzene was the one non-*N*-substituted aromatic compound that induced skin neoplasms, and like rhodamine 6G, benzene was not mutagenic in Salmonella but was clastogenic. Keratoacanthomas occurred in some male rats given 3,3'-dimethoxybenzidine-4,4'-diisocyanate, one of the five *N*-substituted aromatics that induced skin neoplasms, but keratoacanthomas were not the major tumor type induced.

In two-stage skin models of carcinogenesis, activation of protein kinase C by a promoter, such as 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), is considered to be an integral event associated with the promotion and development of skin neoplasms (papillomas or carcinomas). O'Brian and Weinstein (1987) found that rhodamine 6G inhibited rat brain protein kinase C after activation with the tumor promoter TPA, presumably through a chemical-lipid interaction and the induction of cytotoxicity, but not in the absence of lipid cofactor. There is no reported evidence that rhodamine 6G inhibits protein kinase C isolated from epidermal cells. However, inhibition of rat brain protein kinase C in vitro suggests rhodamine 6G should not induce skin neoplasms or promote spontaneously occurring skin neoplasms.

The mean historical control incidence of integumentary system keratoacanthomas in untreated control male F344/N rats is 31/1,936 (1.6%; range, 0/50-7/49). Because of the variable background incidence of keratoacanthomas in F344/N rats, it cannot be concluded with certainty that the incidence of keratoacanthomas in the current studies is related to exposure to rhodamine 6G despite evidence for direct dermal contact and the genotoxicity of rhodamine 6G.

The incidence of pheochromocytomas or malignant pheochromocytomas (combined) of the adrenal gland was marginally increased in high dose female rats. Adrenal medullary neoplasms are relatively common in untreated control female F344/N rats and occur with a variable incidence (99/1,968, 5%; range, 0/50-8/50). Because of the lack of response at the low dose and the variable background incidence of these neoplasms in relation to the increased incidence observed in these studies, it cannot be concluded with certainty that the increased incidence of these neoplasms is related to exposure to rhodamine 6G.

Lampidis et al. (1984) observed that the positively charged dyes rhodamine 6G and a structural analog, rhodamine 123, inhibit heartbeat and kill rat cardiac muscle cells in in vitro primary cultures of tissues from neonatal Sprague Dawley rats; the neutral dyes rhodamine B and rhodamine 116 do not. Cationic, but not neutral, rhodamine dyes inhibit oxidative phosphorylation in isolated mitochondria. In other studies, differences were observed in the accumulation of

rhodamine 6G and rhodamine 123 in cardiac and carcinoma cells. Rhodamine 6G and rhodamine 123 selectively inhibit the in vitro (Summerhayes et al., 1982; Lampidis et al., 1985; Wilkie and Fearon, 1985) and in vivo (Fearon et al., 1987) growth of neoplastic cell lines. Lampidis et al. (1985) attributed the selective inhibition and killing of neoplastic cells to the lipophilic positively charged character of these dyes and the difference in transmembrane potential between normal and neoplastic cells. On this basis, lipophilic positively charged dyes such as rhodamine 6G and rhodamine 123 have been proposed as potential antineoplastic agents.

These studies are not designed for determining antineoplastic activity. However, in consideration of the line of evidence described above, a review of overall benign or malignant tumor incidence indicates decreases in the total number of male rats with malignant neoplasms (control, 39; low dose, 33; high dose, 30) and decreases in the total number of malignant neoplasms (52; 41; 40) (Table A1). The total number of male rats with benign neoplasms and the total number of benign neoplasms were similar in control and exposed animals. No significant negative trends were observed at specific target sites in rats. No differences were observed in female rats for either benign or malignant neoplasms (Table B1).

Both the number of male mice with malignant neoplasms (control, 30; low dose, 25; high dose, 16) and the total number of malignant neoplasms (35; 28; 18) decreased (Table C1). Benign neoplasm incidences did not change. No differences were observed for the total number of animals with neoplasms or total number of benign or malignant neoplasms in female mice (Table D1). However, a decrease in lymphomas (16/50; 8/50; 7/50) was observed. The incidence of these neoplasms in controls is highly variable (5/50-37/50).

The experimental and tabulated data for the NTP Technical Report on rhodamine 6G were examined for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations. As summarized in Appendix H, the audit revealed no major problems with the conduct of the studies or with collection and documentation of the experimental data. No discrepancies were found that influenced the final interpretation of the results of these studies.

Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** for male F344/N rats administered rhodamine 6G, as indicated by a marginally increased incidence of integumentary keratoacanthomas. There was *equivocal evidence of carcinogenic activity* for female F344/N rats administered rhodamine 6G, as indicated by a marginal increase in pheochromocytomas or malignant pheochromocytomas (combined) of the adrenal gland. There was *no evidence of carcinogenic activity* for male B6C3F$_1$ mice administered 1,000 or 2,000 ppm rhodamine 6G in the diet. There was *no evidence of carcinogenic activity* for female B6C3F$_1$ mice administered 500 or 1,000 ppm rhodamine 6G in the diet.

There were no significant nonneoplastic lesions attributed to rhodamine 6G administration to male or female rats or male or female mice. Male and female rats might have been able to tolerate a higher concentration of rhodamine 6G in the feed.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 9.

V. REFERENCES

V. REFERENCES

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Intestine large	(50)	(49)	(50)
Serosa, mesothelioma malignant		1 (2%)	
Intestine small	(50)	(49)	(50)
Ileum, polyp adenomatous		1 (2%)	
Serosa, mesothelioma malignant		1 (2%)	
Liver	(50)	(50)	(50)
Hepatocellular carcinoma	1 (2%)		1 (2%)
Leukemia mononuclear	26 (52%)	20 (40%)	19 (38%)
Neoplastic nodule	3 (6%)		4 (8%)
Neoplastic nodule, multiple	1 (2%)		2 (4%)
Capsule, mesothelioma malignant		1 (2%)	
Mesentery	*(50)	*(50)	*(50)
Mesothelioma malignant	1 (2%)	2 (4%)	1 (2%)
Sarcoma			1 (2%)
Pancreas	(50)	(49)	(50)
Adenoma		1 (2%)	
Leukemia mononuclear	1 (2%)	1 (2%)	2 (4%)
Acinus, adenoma	2 (4%)	4 (8%)	
Acinus, adenoma, multiple		1 (2%)	
Serosa, mesothelioma malignant		1 (2%)	
Salivary glands	(50)	(49)	(50)
Leukemia mononuclear		1 (2%)	
Stomach	(49)	(50)	(50)
Papilloma squamous		1 (2%)	
Serosa, mesothelioma malignant		2 (4%)	
Serosa, sarcoma			1 (2%)
Tongue	*(50)	*(50)	*(50)
Papilloma squamous			1 (2%)
Tooth	*(50)	*(50)	*(50)
Neoplasm, NOS			1 (2%)
CARDIOVASCULAR SYSTEM			
Heart	(50)	(50)	(50)
Leukemia mononuclear	7 (14%)	8 (16%)	5 (10%)
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(49)	(50)
Leukemia mononuclear	6 (12%)	10 (20%)	10 (20%)
Cortex, adenoma	1 (2%)		
Medulla, leukemia mononuclear		1 (2%)	
Medulla, pheochromocytoma malignant	9 (18%)	8 (16%)	5 (10%)
Medulla, pheochromocytoma malignant, multiple	1 (2%)		
Medulla, pheochromocytoma benign	14 (28%)	21 (43%)	19 (38%)
Medulla, pheochromocytoma benign, multiple	4 (8%)	4 (8%)	4 (8%)
Islets, pancreatic	(50)	(49)	(50)
Adenoma	1 (2%)	2 (4%)	4 (8%)
Adenoma, multiple		1 (2%)	
Carcinoma	1 (2%)	1 (2%)	1 (2%)
Pituitary gland	(49)	(49)	(49)
Leukemia mononuclear	4 (8%)	8 (16%)	4 (8%)
Pars distalis, adenoma	9 (18%)	8 (16%)	12 (24%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
Thyroid gland	(50)	(48)	(50)
Leukemia mononuclear	1 (2%)		
C-cell, adenoma	5 (10%)	6 (13%)	1 (2%)
C-cell, carcinoma	3 (6%)	1 (2%)	1 (2%)
Follicular cell, adenoma	1 (2%)		
Follicular cell, carcinoma	1 (2%)		
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Preputial gland	(49)	(48)	(43)
Adenoma	2 (4%)	3 (6%)	2 (5%)
Carcinoma	2 (4%)	4 (8%)	4 (9%)
Prostate	(50)	(49)	(50)
Schwannoma malignant	1 (2%)		
Seminal vesicle	*(50)	*(50)	*(50)
Serosa, mesothelioma malignant		1 (2%)	
Testes	(49)	(50)	(50)
Leukemia mononuclear	1 (2%)		1 (2%)
Seminoma malignant, poor			1 (2%)
Capsule, mesothelioma malignant		2 (4%)	
Interstitial cell, adenoma	3 (6%)	7 (14%)	12 (24%)
Interstitial cell, adenoma, multiple	43 (88%)	42 (84%)	32 (64%)
Tunic, mesothelioma malignant	1 (2%)		
HEMATOPOIETIC SYSTEM			
Blood	*(50)	*(50)	*(50)
Leukemia mononuclear	19 (38%)	17 (34%)	14 (28%)
Bone marrow	(50)	(50)	(50)
Leukemia mononuclear	7 (14%)	17 (34%)	13 (26%)
Lymph node	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)	
Axillary, leukemia mononuclear		1 (2%)	1 (2%)
Iliac, leukemia mononuclear		1 (2%)	1 (2%)
Inguinal, leukemia mononuclear		2 (4%)	
Lumbar, leukemia mononuclear			1 (2%)
Mandibular, leukemia mononuclear	9 (18%)	9 (18%)	3 (6%)
Mediastinal, leukemia mononuclear	2 (4%)	2 (4%)	
Mesenteric, leukemia mononuclear	9 (18%)	6 (12%)	4 (8%)
Pancreatic, leukemia mononuclear	4 (8%)	6 (12%)	
Renal, leukemia mononuclear		2 (4%)	1 (2%)
Spleen	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)		
Leukemia mononuclear	27 (54%)	21 (42%)	19 (38%)
Sarcoma	1 (2%)		
Capsule, mesothelioma malignant		1 (2%)	
Capsule, sarcoma			1 (2%)
Thymus	(47)	(47)	(47)
Leukemia mononuclear	6 (13%)	6 (13%)	1 (2%)
INTEGUMENTARY SYSTEM			
Mammary gland	(50)	(49)	(46)
Fibroadenoma	5 (10%)	1 (2%)	4 (9%)
Fibroadenoma, multiple	1 (2%)		

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
INTEGUMENTARY SYSTEM (Continued)			
Skin	(50)	(50)	(50)
Basal cell adenoma		1 (2%)	2 (4%)
Keratoacanthoma	1 (2%)	2 (4%)	6 (12%)
Keratoacanthoma, multiple			2 (4%)
Leukemia mononuclear	1 (2%)		
Papilloma squamous	2 (4%)		3 (6%)
Trichoepithelioma		1 (2%)	1 (2%)
Subcutaneous tissue, fibroma	4 (8%)	6 (12%)	4 (8%)
Subcutaneous tissue, fibrosarcoma		1 (2%)	1 (2%)
Subcutaneous tissue, liposarcoma	1 (2%)		
Subcutaneous tissue, sarcoma		1 (2%)	
Subcutaneous tissue, schwannoma benign		1 (2%)	
MUSCULOSKELETAL SYSTEM			
Bone	(50)	(50)	(50)
Osteoma			1 (2%)
Osteosarcoma			1 (2%)
Vertebra, chordoma			1 (2%)
Skeletal muscle	*(50)	*(50)	*(50)
Mesothelioma malignant, multiple		1 (2%)	
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Astrocytoma malignant			1 (2%)
Leukemia mononuclear	3 (6%)	7 (14%)	3 (6%)
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)		1 (2%)
Alveolar/bronchiolar carcinoma		1 (2%)	
Leukemia mononuclear	17 (34%)	19 (38%)	17 (34%)
Osteosarcoma, metastatic, multiple, bone			1 (2%)
Nose	(50)	(50)	(45)
Adenocarcinoma	1 (2%)		
Adenocarcinoma, moderately well	1 (2%)		
Papilloma		1 (2%)	
SPECIAL SENSES SYSTEM			
None			
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Leukemia mononuclear	5 (10%)	6 (12%)	4 (8%)
Renal tubule, adenoma			1 (2%)
Renal tubule, carcinoma		1 (2%)	
Urinary bladder	(50)	(49)	(50)
Leukemia mononuclear	1 (2%)	2 (4%)	2 (4%)
Serosa, mesothelioma malignant		2 (4%)	
Transitional epithelium, adenoma		1 (2%)	

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Leukemia mononuclear	27 (54%)	21 (42%)	19 (38%)
Hemangiosarcoma	1 (2%)		
Mesothelioma malignant	1 (2%)	2 (4%)	1 (2%)
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Moribund	23	25	19
Terminal sacrifice	22	20	27
Dead	5	5	4
TUMOR SUMMARY			
Total animals with primary neoplasms **	50	50	49
Total primary neoplasms	156	157	159
Total animals with benign neoplasms	49	50	48
Total benign neoplasms	104	116	118
Total animals with malignant neoplasms	39	33	30
Total malignant neoplasms	52	41	40
Total animals with secondary neoplasms ***		1	1
Total secondary neoplasms		1	1
Total animal neoplasms-- uncertain benign or malignant			1
Total uncertain neoplasms			1

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: UNTREATED CONTROL

WEEKS ON STUDY	082	083	084	086	087	087	089	089	089	095	095	096	096	096	100	100	100	100	100	100	100	100	100	102
CARCASS ID	071	041	081	051	042	072	021	022	052	091	061	082	011	101	043	062	073	102	023	044	092	093	083	012	053
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Leukemia mononuclear	X		X		X	X	X					X		X	X	X		X		X	X		X		X
Neoplastic nodule																									
Neoplastic nodule, multiple																									
Mesentery																									
Mesothelioma malignant																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X																			
Acinus, adenoma																				X					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIOVASCULAR SYSTEM																									
Blood vessel													+												
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X				X								X			X		X					X
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X		X				X											X							X
Cortex, adenoma					X																				
Medulla, pheochromocytoma malignant																	X			X				X	X
Medulla, pheochromocytoma malignant, multiple																			X						
Medulla, pheochromocytoma benign			X					X							X		X			X		X		X	
Medulla, pheochromocytoma benign, multiple											X														
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X	X																		
Pars distalis, adenoma	X	X																							
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X																		
C-cell, adenoma											X								X					X	
C-cell, carcinoma											X														
Follicular cell, adenoma																									
Follicular cell, carcinoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Adenoma								X																	
Carcinoma										X	X														
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant														X											
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Leukemia mononuclear							X																		
Interstitial cell, adenoma				X																					
Interstitial cell, adenoma, multiple	X		X		X	X	X	X	X		X	X	X	X	X	X		X		X	X	X	X	X	X
Tunic, mesothelioma malignant																									

+: Tissue examined microscopically
: Not examined
−: Present but not examined microscopically
I: Insufficient tissue

M: Missing
A: Autolysis precludes examination
X: Incidence of listed morphology

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	104	104	104	105	105	105	105	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	
CARCASS ID	013	063	103	014	024	031	094	015	025	032	033	034	035	045	054	055	064	065	074	075	084	085	095	104	105	TOTAL: TISSUES / TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma											X															1
Leukemia mononuclear			X	X	X	X	X	X				X	X			X			X	X				X		26
Neoplastic nodule						X																	X	X		3
Neoplastic nodule, multiple				X																						1
Mesentery													+						+							2
Mesothelioma malignant																			X							1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Acinus, adenoma																									X	2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CARDIOVASCULAR SYSTEM																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X																							7
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X																							6
Cortex, adenoma																										1
Medulla, pheochromocytoma malignant				X						X					X				X		X					9
Medulla, pheochromocytoma malignant, multiple																										1
Medulla, pheochromocytoma benign			X		X			X				X			X		X		X							14
Medulla, pheochromocytoma benign, multiple						X								X										X		4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X																		1
Carcinoma													X													1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear			X																							4
Pars distalis, adenoma	X	X			X			X									X		X						X	9
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
C-cell, adenoma	X																								X	5
C-cell, carcinoma			X																			X				3
Follicular cell, adenoma									X																	1
Follicular cell, carcinoma																								X		1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma						X																				2
Carcinoma																										2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Interstitial cell, adenoma		X						X																		3
Interstitial cell, adenoma, multiple	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	43
Tunic, mesothelioma malignant																			X							1

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	082	083	084	086	087	087	089	089	089	095	095	096	096	096	100	100	100	100	100	100	100	100	100	100	102
CARCASS ID	071	041	081	051	042	072	021	022	052	091	061	082	011	101	043	062	073	102	023	044	092	093	083	012	053
HEMATOPOIETIC SYSTEM																									
Blood	+	+		+	+	+		+		+	+	+	+		+	+		+	+	+	+	+	+	+	
Leukemia mononuclear	X				X	X						X			X			X			X		X		
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X								X	X		X							X
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mandibular, leukemia mononuclear			X		X	X	X					X			X			X							X
Mediastinal, leukemia mononuclear			X									X													
Mesenteric, leukemia mononuclear			X		X	X	X					X			X			X							X
Pancreatic, leukemia mononuclear			X			X						X													X
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Leukemia mononuclear	X		X		X	X	X				X	X		X	X	X		X		X	X		X		X
Sarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X	X								X			X							X
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																				X					
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma												X													
Leukemia mononuclear							X																		
Papilloma squamous																			X				X		
Subcutaneous tissue, fibroma											X						X								
Subcutaneous tissue, liposarcoma																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X				X								X										
Spinal cord								+																	
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma							X														X				
Leukemia mononuclear	X		X		X	X	X					X			X	X		X		X					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma		X																							
Adenocarcinoma, moderately well				X																					
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Ear																		+							
Eye		+		+				+																	
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X		X	X	X											X							
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	104	104	104	105	105	105	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	TOTAL:
CARCASS ID	013	063	103	014	024	031	094	015	025	032	033	034	035	045	054	055	064	065	074	075	084	085	095	104	105	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Blood	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Leukemia mononuclear			X	X	X	X	X	X				X	X			X			X					X		19
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X																					X		7
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mandibular, leukemia mononuclear			X																							9
Mediastinal, leukemia mononuclear																										2
Mesenteric, leukemia mononuclear			X																							9
Pancreatic, leukemia mononuclear																										4
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma		X																								1
Leukemia mononuclear			X	X	X	X	X	X				X	X			X			X	X				X		27
Sarcoma						X																				1
Thymus	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leukemia mononuclear			X																							6
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma						X	X					X													X	5
Fibroadenoma, multiple																		X								1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																										1
Leukemia mononuclear																										1
Papilloma squamous																										2
Subcutaneous tissue, fibroma								X								X										4
Subcutaneous tissue, liposarcoma							X																			1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										3
Spinal cord																										1
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										2
Leukemia mononuclear			X	X		X		X								X			X					X		17
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																										1
Adenocarcinoma, moderately well																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Ear	+	+			+		+	+		+					+		+	+		+	+		+	+		14
Eye	+																									4
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										5
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X																							1

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: LOW DOSE

WEEKS ON STUDY	075	077	080	084	089	089	089	092	092	093	093	093	093	094	095	095	095	096	099	099	100	100	100	100	102
CARCASS ID	241	291	301	302	251	211	281	282	292	261	271	212	283	231	221	232	222	213	214	242	252	223	293	233	294
ALIMENTARY SYSTEM																									
Esophagus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serosa, mesothelioma malignant		X																							
Intestine small	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ileum, polyp adenomatous															X										
Serosa, mesothelioma malignant		X																							
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X				X	X		X	X	X	X	X			X		X				X	X	X
Capsule, mesothelioma malignant		X																							
Mesentery	+	+														+									
Mesothelioma malignant	X	X																							
Pancreas	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X					
Leukemia mononuclear																			X						
Acinus, adenoma																									
Acinus, adenoma, multiple																									
Serosa, mesothelioma malignant		X																							
Salivary glands	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																			X						
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																				X					
Serosa, mesothelioma malignant	X	X																							
CARDIOVASCULAR SYSTEM																									
Blood vessel									+																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X					X	X	X	X		X		X						
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X				X	X	X	X		X		X						
Medulla, leukemia mononuclear											X														
Medulla, pheochromocytoma malignant									X							X			X					X	
Medulla, pheochromocytoma benign								X		X	X			X		X	X		X			X		X	X
Medulla, pheochromocytoma benign, multiple																					X				
Islets, pancreatic	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adenoma, multiple																									
Carcinoma																									
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	M	+	+	+
Pituitary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear															X		X		X				X	X	
Pars distalis, adenoma	X													X			X			X					
Thyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
C-cell, adenoma				X																					
C-cell, carcinoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma					X										X	X						X			
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Seminal vesicle		+			+						+														
Serosa, mesothelioma malignant		X																							
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, mesothelioma malignant	X	X																		X					
Interstitial cell, adenoma			X				X	X	X																
Interstitial cell, adenoma, multiple	X	X		X	X	X				X	X	X	X		X	X	X	X	X		X	X	X	X	X

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

WEEKS ON STUDY	103	104	104	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	262	243	272	273	274	215	234	284	224	225	235	244	245	253	254	255	263	264	265	275	285	295	303	304	305	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Serosa, mesothelioma malignant																										1
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ileum, polyp adenomatous																										1
Serosa, *mesothelioma malignant*																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X			X	X	X														X		X		20
Capsule, mesothelioma malignant																										1
Mesentery									+																	4
Mesothelioma malignant																										2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Leukemia mononuclear																										1
Acinus, adenoma					X		X								X								X			4
Acinus, adenoma, multiple																X										1
Serosa, mesothelioma malignant																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papilloma squamous																										1
Serosa, mesothelioma malignant																										2
CARDIOVASCULAR SYSTEM																										
Blood vessel					+																					2
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear						X																				8
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear	X					X																		X		10
Medulla, leukemia mononuclear																										1
Medulla, *pheochromocytoma malignant*		X										X		X								X				8
Medulla, pheochromocytoma benign		X	X				X		X			X	X		X				X				X		X	21
Medulla, pheochromocytoma benign, multiple														X						X	X					4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma											X					X										2
Adenoma, multiple	X																									1
Carcinoma													X													1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear			X					X														X				8
Pars distalis, adenoma												X				X	X								X	8
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma	X															X	X				X	X				6
C-cell, carcinoma				X																						1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma													X			X			X							3
Carcinoma																										4
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle												+														4
Serosa, mesothelioma malignant																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, mesothelioma malignant																										2
Interstitial cell, adenoma									X			X														7
Interstitial cell, adenoma, multiple	X	X	X	X	X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	42

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

WEEKS ON STUDY	075	077	080	084	089	089	089	092	092	093	093	093	093	094	095	095	095	096	099	099	100	100	100	100	102
CARCASS ID	241	291	301	302	251	211	281	282	292	261	271	212	283	231	221	232	222	213	214	242	252	223	293	233	294
HEMATOPOIETIC SYSTEM																									
Blood		+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X	X		X	X	X	X	X	X		X		X				X		X
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X	X		X	X	X	X	X	X		X		X				X	X	
Lymph node	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																		X							
Axillary, leukemia mononuclear							X																		
Iliac, leukemia mononuclear							X																		
Inguinal, leukemia mononuclear																			X						
Mandibular, leukemia mononuclear							X				X	X	X				X		X						
Mediastinal, leukemia mononuclear							X						X												
Mesenteric, leukemia mononuclear							X				X	X	X						X						
Pancreatic, leukemia mononuclear							X					X	X						X						
Renal, leukemia mononuclear							X																		
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X				X	X		X	X	X	X	X	X		X		X				X	X	X
Capsule, mesothelioma malignant	X																								
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+
Leukemia mononuclear							X				X	X					X		X						
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma																X									
Trichoepithelioma																									
Subcutaneous tissue, fibroma						X																			X
Subcutaneous tissue, fibrosarcoma																								X	
Subcutaneous tissue, sarcoma				X																					
Subcutaneous tissue, schwannoma benign																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle		+																							
Mesothelioma malignant, multiple		X																							
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear												X					X		X				X		
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																		X							
Leukemia mononuclear			X				X	X		X	X	X	X	X	X		X		X				X	X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma																									
Trachea	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Ear												+													
Eye				+								+				+									
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X					X	X	X					X						
Renal tubule, carcinoma																								X	
Urinary bladder	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear											X								X						
Serosa, mesothelioma malignant	X	X																							
Transitional epithelium, adenoma																									

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

WEEKS ON STUDY	103	104	104	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	262	243	272	273	274	215	234	284	224	225	235	244	245	253	254	255	263	264	265	275	285	295	303	304	305		TISSUES TUMORS
HEMATOPOIETIC SYSTEM																											
Blood	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Leukemia mononuclear	X		X			X		X																X			17
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia mononuclear	X		X			X		X																X			17
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Alveolar/bronchiolar carcinoma, metastatic, lung																											1
Axillary, leukemia mononuclear																											1
Iliac, leukemia mononuclear																											1
Inguinal, leukemia mononuclear						X																					2
Mandibular, leukemia mononuclear			X			X		X																			9
Mediastinal, leukemia mononuclear																											2
Mesenteric, leukemia mononuclear						X																					6
Pancreatic, leukemia mononuclear	X					X																					6
Renal, leukemia mononuclear						X																					2
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia mononuclear	X		X			X	X	X														X		X			21
Capsule, mesothelioma malignant																											1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+		47
Leukemia mononuclear						X																					6
INTEGUMENTARY SYSTEM																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Fibroadenoma											X																1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Basal cell adenoma																							X				1
Keratoacanthoma																						X					2
Trichoepithelioma											X																1
Subcutaneous tissue, fibroma							X				X		X										X				6
Subcutaneous tissue, fibrosarcoma																											1
Subcutaneous tissue, sarcoma																											1
Subcutan. tissue, schwannoma benign												X															1
MUSCULOSKELETAL SYSTEM																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Skeletal muscle																			+								2
Mesothelioma malignant, multiple																											1
NERVOUS SYSTEM																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia mononuclear			X					X														X					7
RESPIRATORY SYSTEM																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar carcinoma																											1
Leukemia mononuclear	X		X			X		X														X		X			19
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Papilloma													X														1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
SPECIAL SENSES SYSTEM																											
Ear					+		+			+					+						+	+			+	8
Eye					+		+			+			+		+												8
URINARY SYSTEM																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia mononuclear						X																					6
Renal tubule, carcinoma																											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Leukemia mononuclear																											2
Serosa, mesothelioma malignant																											2
Transitional epithelium, adenoma											X																1

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: HIGH DOSE

WEEKS ON STUDY	053	065	067	069	083	090	092	093	094	094	094	095	096	098	100	100	100	100	102	103	104	104	105	105
CARCASS ID	181	151	141	161	191	201	131	121	111	152	153	182	192	188	162	171	122	142	132	112	163	202	113	114	115
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Leukemia mononuclear		X	X		X	X			X		X	X	X						X	X	X				
Neoplastic nodule														X									X		
Neoplastic nodule, multiple																									
Mesentery								+	+																
Mesothelioma malignant																									
Sarcoma								X																	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X							X												
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serosa, sarcoma																									
Tongue			+																						
Papilloma squamous			X																						
Tooth																						+			
Neoplasm, NOS																						X			
CARDIOVASCULAR SYSTEM																									
Blood vessel							+																		
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X									X	X	X												
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X			X						X	X	X						X	X					
Medulla, pheochromocytoma malignant																									
Medulla, pheochromocytoma benign						X		X					X			X	X		X	X	X		X		X
Medulla, pheochromocytoma benign, multiple														X											
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X																		
Carcinoma																			X						
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Leukemia mononuclear		X				X						X	X												
Pars distalis, adenoma				X						X					X						X				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
C-cell, carcinoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	M	M	+	M	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X					X																	
Carcinoma													X					X							
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle																		+							
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X																			
Seminoma malignant, poor							X																		
Interstitial cell, adenoma			X		X								X		X					X		X	X		
Interstitial cell, adenoma, multiple				X				X	X		X	X		X		X	X	X	X		X			X	X

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
CARCASS ID	123	124	125	133	134	135	143	144	145	154	155	164	165	172	173	174	175	184	185	193	194	195	203	204	205	TOTAL: TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma							X																			1
Leukemia mononuclear							X	X		X							X		X	X			X	X		19
Neoplastic nodule																			X					X		4
Neoplastic nodule, multiple							X														X					2
Mesentery						+																	+			4
Mesothelioma malignant						X																				1
Sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Serosa, sarcoma																		X								1
Tongue													+													2
Papilloma squamous																										1
Tooth																										1
Neoplasm, NOS																										1
CARDIOVASCULAR SYSTEM																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																								X		5
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear							X										X							X		10
Medulla, pheochromocytoma malignant						X			X							X						X		X		5
Medulla, pheochromocytoma benign							X	X	X			X						X		X			X	X	X	19
Medulla, pheochromocytoma benign, multiple													X				X		X							4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X												X							X					4
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										4
Pars distalis, adenoma	X	X			X		X			X		X			X							X				12
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																		X								1
C-cell, carcinoma						X																				1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	43
Adenoma																										2
Carcinoma														X									X			4
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+																									2
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Seminoma malignant, poor																										1
Interstitial cell, adenoma	X	X					X			X											X					12
Interstitial cell, adenoma, multiple			X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X			X	X	X	32

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

WEEKS ON STUDY	053	065	067	069	083	090	092	093	094	094	094	095	096	098	100	100	100	100	100	102	103	104	104	105	105
CARCASS ID	181	151	141	161	191	201	131	121	111	152	153	182	192	183	162	171	122	142	132	112	163	202	113	114	115
HEMATOPOIETIC SYSTEM																									
Blood		+	+	+		+	+		+	+	+	+	+			+	+	+	+	+		+	+	+	+
Leukemia mononuclear		X				X			X			X	X						X	X					
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Leukemia mononuclear		X	X						X		X	X							X		X				
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Axillary, leukemia mononuclear			X																						
Iliac, leukemia mononuclear																									
Lumbar, leukemia mononuclear																									
Mandibular, leukemia mononuclear		X							X																
Mesenteric, leukemia mononuclear		X			X	X							X												
Renal, leukemia mononuclear			X																						
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	X		X	X			X		X	X	X						X	X	X				
Capsule, sarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Fibroadenoma														X											
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																	X								
Keratoacanthoma													X	X					X						
Keratoacanthoma, multiple																				X					
Papilloma squamous						X																			
Trichoepithelioma																		X							
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibrosarcoma							X																		
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteoma																							X		
Osteosarcoma										X															
Vertebra, chordoma				X																					
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant								X																	
Leukemia mononuclear		X				X						X													
Spinal cord				+						+												+			
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Leukemia mononuclear		X	X		X	X			X		X	X	X						X	X	X				
Osteosarcoma, metastatic, multiple, bone										X															
Nose	M	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Ear							+															+		+	+
Eye			+							+	+								+					+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X						X													
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X																							

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	123	124	125	133	134	135	143	144	145	154	155	164	165	172	173	174	175	184	185	193	194	195	203	204	205		TISSUES TUMORS
HEMATOPOIETIC SYSTEM																											
Blood	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		44
Leukemia mononuclear							X	X		X							X		X	X				X			14
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia mononuclear							X	X		X							X			X				X			13
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Axillary, leukemia mononuclear																											1
Iliac, leukemia mononuclear																								X			1
Lumbar, leukemia mononuclear																								X			1
Mandibular, leukemia mononuclear																								X			3
Mesenteric, leukemia mononuclear																											4
Renal, leukemia mononuclear																											1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia mononuclear							X	X		X							X		X	X			X	X			19
Capsule, sarcoma																		X									1
Thymus	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Leukemia mononuclear																								X			1
INTEGUMENTARY SYSTEM																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M		46
Fibroadenoma			X		X																			X			4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Basal cell adenoma			X																								2
Keratoacanthoma										X		X									X						6
Keratoacanthoma, multiple						X																					2
Papilloma squamous														X										X			3
Trichoepithelioma																											1
Subcutaneous tissue, fibroma						X		X							X				X								4
Subcutaneous tissue, fibrosarcoma																											1
MUSCULOSKELETAL SYSTEM																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Osteoma																											1
Osteosarcoma																											1
Vertebra, chordoma																											1
NERVOUS SYSTEM																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Astrocytoma malignant																											1
Leukemia mononuclear																											3
Spinal cord																											3
RESPIRATORY SYSTEM																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Alveolar/bronchiolar adenoma															X												1
Leukemia mononuclear							X	X		X										X			X	X			17
Osteosarcoma, metastatic, multiple, bone																											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+		45
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
SPECIAL SENSES SYSTEM																											
Ear	+		+	+	+	+					+	+		+	+		+					+		+	+	17
Eye	+	+		+	+	+		+	+	+	+			+		+	+										18
URINARY SYSTEM																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia mononuclear																								X			4
Renal tubule, adenoma	X																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Leukemia mononuclear																								X			2

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Control	120 ppm	250 ppm
Adrenal Medulla: Pheochromocytoma			
Overall Rates (a)	18/50 (36%)	25/49 (51%)	23/50 (46%)
Adjusted Rates (b)	55.0%	72.0%	61.3%
Terminal Rates (c)	9/22 (41%)	12/21 (57%)	13/27 (48%)
Day of First Observation	585	639	629
Life Table Tests (d)	P=0.435	P=0.101	P=0.435
Logistic Regression Tests (d)	P=0.185	P=0.081	P=0.203
Cochran-Armitage Trend Test (d)	P=0.191		
Fisher Exact Test (d)		P=0.096	P=0.208
Adrenal Medulla: Malignant Pheochromocytoma			
Overall Rates (a)	10/50 (20%)	8/49 (16%)	5/50 (10%)
Adjusted Rates (b)	34.8%	27.3%	18.5%
Terminal Rates (c)	5/22 (23%)	3/21 (14%)	5/27 (19%)
Day of First Observation	694	643	733
Life Table Tests (d)	P=0.065N	P=0.459N	P=0.072N
Logistic Regression Tests (d)	P=0.090N	P=0.432N	P=0.097N
Cochran-Armitage Trend Test (d)	P=0.107N		
Fisher Exact Test (d)		P=0.416N	P=0.131N
Adrenal Medulla: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	23/50 (46%)	27/49 (55%)	26/50 (52%)
Adjusted Rates (b)	67.8%	75.7%	69.6%
Terminal Rates (c)	12/22 (55%)	13/21 (62%)	16/27 (59%)
Day of First Observation	585	639	629
Life Table Tests (d)	P=0.463N	P=0.228	P=0.513N
Logistic Regression Tests (d)	P=0.315	P=0.209	P=0.352
Cochran-Armitage Trend Test (d)	P=0.316		
Fisher Exact Test (d)		P=0.241	P=0.345
Preputial Gland: Adenoma			
Overall Rates (a)	2/49 (4%)	3/48 (6%)	2/43 (5%)
Adjusted Rates (b)	6.8%	14.3%	4.4%
Terminal Rates (c)	1/22 (5%)	3/21 (14%)	0/25 (0%)
Day of First Observation	620	733	468
Life Table Tests (d)	P=0.554N	P=0.487	P=0.676N
Logistic Regression Tests (d)	P=0.515	P=0.485	P=0.636
Cochran-Armitage Trend Test (d)	P=0.547		
Fisher Exact Test (d)		P=0.490	P=0.641
Preputial Gland: Carcinoma			
Overall Rates (a)	2/49 (4%)	4/48 (8%)	4/43 (9%)
Adjusted Rates (b)	4.9%	10.8%	13.1%
Terminal Rates (c)	0/22 (0%)	0/21 (0%)	2/25 (8%)
Day of First Observation	661	619	667
Life Table Tests (d)	P=0.310	P=0.298	P=0.367
Logistic Regression Tests (d)	P=0.196	P=0.337	P=0.265
Cochran-Armitage Trend Test (d)	P=0.222		
Fisher Exact Test (d)		P=0.329	P=0.278
Preputial Gland: Adenoma or Carcinoma			
Overall Rates (a)	4/49 (8%)	7/48 (15%)	6/43 (14%)
Adjusted Rates (b)	11.3%	23.5%	16.8%
Terminal Rates (c)	1/22 (5%)	3/21 (14%)	2/25 (8%)
Day of First Observation	620	619	468
Life Table Tests (d)	P=0.375	P=0.235	P=0.407
Logistic Regression Tests (d)	P=0.198	P=0.253	P=0.217
Cochran-Armitage Trend Test (d)	P=0.245		
Fisher Exact Test (d)		P=0.250	P=0.289

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Control	120 ppm	250 ppm
Pancreatic Islets: Adenoma			
Overall Rates (a)	1/50 (2%)	3/49 (6%)	4/50 (8%)
Adjusted Rates (b)	4.5%	13.1%	13.1%
Terminal Rates (c)	1/22 (5%)	2/21 (10%)	3/27 (11%)
Day of First Observation	733	720	643
Life Table Tests (d)	P=0.196	P=0.293	P=0.237
Logistic Regression Tests (d)	P=0.155	P=0.289	P=0.187
Cochran-Armitage Trend Test (d)	P=0.137		
Fisher Exact Test (d)		P=0.301	P=0.181
Pancreatic Islets: Adenoma or Carcinoma			
Overall Rates (a)	2/50 (4%)	4/49 (8%)	5/50 (10%)
Adjusted Rates (b)	9.1%	17.7%	15.9%
Terminal Rates (c)	2/22 (9%)	3/21 (14%)	3/27 (11%)
Day of First Observation	733	720	643
Life Table Tests (d)	P=0.255	P=0.317	P=0.296
Logistic Regression Tests (d)	P=0.200	P=0.318	P=0.230
Cochran-Armitage Trend Test (d)	P=0.173		
Fisher Exact Test (d)		P=0.329	P=0.218
Liver: Neoplastic Nodule			
Overall Rates (a)	4/50 (8%)	0/50 (0%)	6/50 (12%)
Adjusted Rates (b)	18.2%	0.0%	20.7%
Terminal Rates (c)	4/22 (18%)	0/21 (0%)	5/27 (19%)
Day of First Observation	733		680
Life Table Tests (d)	P=0.352	P=0.066N	P=0.491
Logistic Regression Tests (d)	P=0.315	P=0.066N	P=0.443
Cochran-Armitage Trend Test (d)	P=0.259		
Fisher Exact Test (d)		P=0.059N	P=0.370
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall Rates (a)	5/50 (10%)	0/50 (0%)	6/50 (12%)
Adjusted Rates (b)	22.7%	0.0%	20.7%
Terminal Rates (c)	5/22 (23%)	0/21 (0%)	5/27 (19%)
Day of First Observation	733		680
Life Table Tests (d)	P=0.517	P=0.034N	P=0.626N
Logistic Regression Tests (d)	P=0.477	P=0.034N	P=0.588
Cochran-Armitage Trend Test (d)	P=0.405		
Fisher Exact Test (d)		P=0.028N	P=0.500
Mammary Gland: Fibroadenoma			
Overall Rates (a)	6/50 (12%)	1/50 (2%)	4/50 (8%)
Adjusted Rates (b)	25.1%	4.8%	13.5%
Terminal Rates (c)	5/22 (23%)	1/21 (5%)	3/27 (11%)
Day of First Observation	695	733	680
Life Table Tests (d)	P=0.216N	P=0.064N	P=0.263N
Logistic Regression Tests (d)	P=0.251N	P=0.061N	P=0.314N
Cochran-Armitage Trend Test (d)	P=0.299N		
Fisher Exact Test (d)		P=0.056N	P=0.370N
Pancreas: Adenoma			
Overall Rates (a)	2/50 (4%)	(e) 6/49 (12%)	0/50 (0%)
Adjusted Rates (b)	7.5%	26.2%	0.0%
Terminal Rates (c)	1/22 (5%)	5/21 (24%)	0/27 (0%)
Day of First Observation	695	692	
Life Table Tests (d)	P=0.182N	P=0.114	P=0.208N
Logistic Regression Tests (d)	P=0.206N	P=0.114	P=0.231N
Cochran-Armitage Trend Test (d)	P=0.237N		
Fisher Exact Test (d)		P=0.128	P=0.247N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Control	120 ppm	250 ppm
Pituitary Gland/Pars Distalis: Adenoma			
Overall Rates (a)	9/49 (18%)	8/49 (16%)	12/49 (24%)
Adjusted Rates (b)	31.8%	27.4%	36.9%
Terminal Rates (c)	5/22 (23%)	4/21 (19%)	8/27 (30%)
Day of First Observation	574	522	482
Life Table Tests (d)	P=0.381	P=0.553N	P=0.446
Logistic Regression Tests (d)	P=0.254	P=0.496N	P=0.303
Cochran-Armitage Trend Test (d)	P=0.259		
Fisher Exact Test (d)		P=0.500N	P=0.312
Skin: Keratoacanthoma			
Overall Rates (a)	1/50 (2%)	2/50 (4%)	8/50 (16%)
Adjusted Rates (b)	2.6%	7.5%	24.3%
Terminal Rates (c)	0/22 (0%)	1/21 (5%)	4/27 (15%)
Day of First Observation	667	662	667
Life Table Tests (d)	P=0.013	P=0.460	P=0.033
Logistic Regression Tests (d)	P=0.006	P=0.503	P=0.018
Cochran-Armitage Trend Test (d)	P=0.006		
Fisher Exact Test (d)		P=0.500	P=0.015
Skin: Squamous Papilloma			
Overall Rates (a)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted Rates (b)	6.6%	0.0%	9.5%
Terminal Rates (c)	0/22 (0%)	0/21 (0%)	2/27 (7%)
Day of First Observation	695		629
Life Table Tests (d)	P=0.443	P=0.259N	P=0.570
Logistic Regression Tests (d)	P=0.379	P=0.240N	P=0.498
Cochran-Armitage Trend Test (d)	P=0.380		
Fisher Exact Test (d)		P=0.247N	P=0.500
Skin: Trichoepithelioma or Basal Cell Adenoma			
Overall Rates (a)	0/50 (0%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	0.0%	9.5%	9.4%
Terminal Rates (c)	0/22 (0%)	2/21 (10%)	1/27 (4%)
Day of First Observation		733	698
Life Table Tests (d)	P=0.127	P=0.227	P=0.160
Logistic Regression Tests (d)	P=0.095	P=0.227	P=0.122
Cochran-Armitage Trend Test (d)	P=0.085		
Fisher Exact Test (d)		P=0.247	P=0.121
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	4/50 (8%)	6/50 (12%)	4/50 (8%)
Adjusted Rates (b)	13.8%	23.9%	14.8%
Terminal Rates (c)	2/22 (9%)	4/21 (19%)	4/27 (15%)
Day of First Observation	662	620	733
Life Table Tests (d)	P=0.456N	P=0.338	P=0.559N
Logistic Regression Tests (d)	P=0.538N	P=0.349	P=0.616N
Cochran-Armitage Trend Test (d)	P=0.564N		
Fisher Exact Test (d)		P=0.370	P=0.643N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	4/50 (8%)	7/50 (14%)	5/50 (10%)
Adjusted Rates (b)	13.8%	26.7%	16.8%
Terminal Rates (c)	2/22 (9%)	4/21 (19%)	4/27 (15%)
Day of First Observation	662	620	643
Life Table Tests (d)	P=0.558	P=0.235	P=0.587
Logistic Regression Tests (d)	P=0.457	P=0.243	P=0.508
Cochran-Armitage Trend Test (d)	P=0.447		
Fisher Exact Test (d)		P=0.262	P=0.500

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Control	120 ppm	250 ppm
Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates (a)	4/50 (8%)	8/50 (16%)	5/50 (10%)
Adjusted Rates (b)	13.8%	28.3%	16.8%
Terminal Rates (c)	2/22 (9%)	4/21 (19%)	4/27 (15%)
Day of First Observation	662	583	643
Life Table Tests (d)	P=0.557	P=0.161	P=0.587
Logistic Regression Tests (d)	P=0.450	P=0.172	P=0.508
Cochran-Armitage Trend Test (d)	P=0.451		
Fisher Exact Test (d)		P=0.178	P=0.500
Testis: Interstitial Cell Adenoma			
Overall Rates (a)	46/49 (94%)	49/50 (98%)	44/50 (88%)
Adjusted Rates (b)	100.0%	100.0%	97.8%
Terminal Rates (c)	22/22 (100%)	21/21 (100%)	26/27 (96%)
Day of First Observation	574	522	468
Life Table Tests (d)	P=0.126N	P=0.267	P=0.137N
Logistic Regression Tests (d)	P=0.302N	P=0.250	P=0.423N
Cochran-Armitage Trend Test (d)	P=0.157N		
Fisher Exact Test (d)		P=0.301	P=0.254N
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	5/50 (10%)	6/48 (13%)	1/50 (2%)
Adjusted Rates (b)	16.7%	23.9%	3.7%
Terminal Rates (c)	1/22 (5%)	4/21 (19%)	1/27 (4%)
Day of First Observation	662	583	733
Life Table Tests (d)	P=0.071N	P=0.463	P=0.083N
Logistic Regression Tests (d)	P=0.094N	P=0.462	P=0.101N
Cochran-Armitage Trend Test (d)	P=0.096N		
Fisher Exact Test (d)		P=0.471	P=0.102N
Thyroid Gland: C-Cell Carcinoma			
Overall Rates (a)	3/50 (6%)	1/48 (2%)	1/50 (2%)
Adjusted Rates (b)	10.7%	4.5%	3.7%
Terminal Rates (c)	1/22 (5%)	0/21 (0%)	1/27 (4%)
Day of First Observation	662	729	733
Life Table Tests (d)	P=0.180N	P=0.332N	P=0.265N
Logistic Regression Tests (d)	P=0.190N	P=0.326N	P=0.296N
Cochran-Armitage Trend Test (d)	P=0.210N		
Fisher Exact Test (d)		P=0.324N	P=0.309N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	7/50 (14%)	7/48 (15%)	2/50 (4%)
Adjusted Rates (b)	23.9%	27.4%	7.4%
Terminal Rates (c)	2/22 (9%)	4/21 (19%)	2/27 (7%)
Day of First Observation	662	583	733
Life Table Tests (d)	P=0.048N	P=0.569	P=0.057N
Logistic Regression Tests (d)	P=0.064N	P=0.568	P=0.072N
Cochran-Armitage Trend Test (d)	P=0.071N		
Fisher Exact Test (d)		P=0.581	P=0.080N
Hematopoietic System: Mononuclear Leukemia			
Overall Rates (a)	27/50 (54%)	21/50 (42%)	19/50 (38%)
Adjusted Rates (b)	68.3%	53.1%	47.1%
Terminal Rates (c)	11/22 (50%)	5/21 (24%)	8/27 (30%)
Day of First Observation	574	559	453
Life Table Tests (d)	P=0.061N	P=0.290N	P=0.065N
Logistic Regression Tests (d)	P=0.060N	P=0.146N	P=0.072N
Cochran-Armitage Trend Test (d)	P=0.068N		
Fisher Exact Test (d)		P=0.158N	P=0.080N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors 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. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) Five tumors diagnosed as pancreas, acinus, adenoma; one tumor diagnosed as pancreas, adenoma

TABLE A4. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM KERATOACANTHOMAS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls
Historical Incidence at Southern Research Institute	
HC Blue No. 2	3/50
C.I. Disperse Blue 1	7/49
Eugenol	0/40
Stannous chloride	0/50
D-Mannitol	0/50
Ziram	1/50
Propyl gallate	0/50
Zearalenone	0/50
HC Blue No. 1	1/50
TOTAL	12/439 (2.7%)
SD (b)	4.78%
Range (c)	
High	7/49
Low	0/50
Overall Historical Incidence	
TOTAL	31/1,936 (1.6%)
SD (b)	2.98%
Range (c)	
High	7/49
Low	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Intestine large	(50)	(49)	(50)
Cecum, inflammation, subacute			2 (4%)
Cecum, parasite metazoan		1 (2%)	
Colon, cyst	1 (2%)		
Colon, diverticulum	1 (2%)		
Colon, edema	1 (2%)		
Colon, parasite metazoan	6 (12%)	5 (10%)	1 (2%)
Rectum, mineralization	1 (2%)	1 (2%)	
Rectum, parasite metazoan	1 (2%)	4 (8%)	
Intestine small	(50)	(49)	(50)
Peyer's patch, mineralization, focal		1 (2%)	
Peyer's patch, necrosis, focal		1 (2%)	
Liver	(50)	(50)	(50)
Angiectasis, focal	7 (14%)	6 (12%)	8 (16%)
Angiectasis, multifocal	8 (16%)	2 (4%)	6 (12%)
Basophilic focus	2 (4%)	4 (8%)	5 (10%)
Basophilic focus, multiple	3 (6%)	1 (2%)	2 (4%)
Clear cell focus			1 (2%)
Congestion		3 (6%)	
Degeneration, fatty, focal			1 (2%)
Developmental malformation	5 (10%)	1 (2%)	5 (10%)
Eosinophilic focus		2 (4%)	
Eosinophilic focus, multiple			1 (2%)
Fibrosis, focal	1 (2%)		
Granuloma, focal			1 (2%)
Granuloma, multifocal	2 (4%)	2 (4%)	2 (4%)
Hematopoietic cell proliferation		1 (2%)	
Hemorrhage, focal	1 (2%)		
Hemorrhage, multifocal		1 (2%)	
Hepatodiaphragmatic nodule		1 (2%)	
Necrosis, focal			1 (2%)
Necrosis, multifocal	1 (2%)	1 (2%)	1 (2%)
Pigmentation, hemosiderin, focal	1 (2%)		
Pigmentation, hemosiderin, multifocal		1 (2%)	
Thrombus		1 (2%)	
Vacuolization cytoplasmic, diffuse	3 (6%)	3 (6%)	5 (10%)
Vacuolization cytoplasmic, focal	1 (2%)	3 (6%)	
Vacuolization cytoplasmic, multifocal	1 (2%)	1 (2%)	
Biliary tract, hyperplasia	32 (64%)	37 (74%)	39 (78%)
Centrilobular, necrosis	12 (24%)	19 (38%)	13 (26%)
Hepatocyte, hypertrophy	1 (2%)	8 (16%)	4 (8%)
Mesentery	(2)	(4)	(4)
Hemorrhage			2 (50%)
Inflammation, subacute, diffuse			1 (25%)
Fat, necrosis, focal	2 (100%)	2 (50%)	2 (50%)
Pancreas	(50)	(49)	(50)
Atrophy	9 (18%)	18 (37%)	11 (22%)
Acinus, hyperplasia		1 (2%)	
Artery, hypertrophy			1 (2%)
Artery, inflammation, subacute	3 (6%)	2 (4%)	1 (2%)
Salivary glands	(50)	(49)	(50)
Infiltration cellular, lymphocytic, multifocal			1 (2%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
ALIMENTARY SYSTEM (Continued)			
Stomach	(49)	(50)	(50)
Inflammation, subacute			1 (2%)
Artery, inflammation, subacute	1 (2%)	1 (2%)	
Forestomach, edema		1 (2%)	
Forestomach, foreign body	1 (2%)		
Forestomach, hyperkeratosis	1 (2%)	2 (4%)	4 (8%)
Forestomach, hyperplasia	1 (2%)	2 (4%)	4 (8%)
Forestomach, inflammation, granulomatous, focal	1 (2%)		
Forestomach, inflammation, subacute	2 (4%)	2 (4%)	2 (4%)
Forestomach, mineralization	1 (2%)	2 (4%)	
Forestomach, ulcer	3 (6%)	1 (2%)	1 (2%)
Glandular, edema		1 (2%)	
Glandular, erosion		1 (2%)	1 (2%)
Glandular, erosion, multiple		1 (2%)	
Glandular, mineralization	1 (2%)	5 (10%)	1 (2%)
Glandular, ulcer	1 (2%)		
Glandular, ulcer, multiple	1 (2%)		
CARDIOVASCULAR SYSTEM			
Blood vessel	(1)	(2)	(1)
Mineralization	1 (100%)	2 (100%)	1 (100%)
Heart	(50)	(50)	(50)
Bacterium		1 (2%)	
Fibrosis, multifocal	35 (70%)	40 (80%)	40 (80%)
Inflammation, suppurative, acute		1 (2%)	
Mineralization	1 (2%)	2 (4%)	1 (2%)
Atrium, thrombus	3 (6%)	3 (6%)	2 (4%)
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(49)	(50)
Hyperplasia, focal		1 (2%)	
Cortex, angiectasis, focal	2 (4%)		
Cortex, congestion	1 (2%)		
Cortex, degeneration, fatty, diffuse			1 (2%)
Cortex, degeneration, fatty, focal	1 (2%)	5 (10%)	6 (12%)
Cortex, degeneration, fatty, multifocal	2 (4%)		1 (2%)
Cortex, hemorrhage, multifocal			1 (2%)
Cortex, hyperplasia, focal			2 (4%)
Cortex, hyperplasia, multifocal	1 (2%)		
Cortex, hyperplasia, multifocal, multifocal	1 (2%)		
Cortex, necrosis, diffuse			1 (2%)
Cortex, necrosis, multifocal			2 (4%)
Medulla, hematopoietic cell proliferation		1 (2%)	2 (4%)
Medulla, hyperplasia, focal	6 (12%)	10 (20%)	2 (4%)
Medulla, hyperplasia, multifocal	1 (2%)	1 (2%)	1 (2%)
Medulla, necrosis, diffuse			1 (2%)
Medulla, necrosis, multifocal	1 (2%)		
Islets, pancreatic	(50)	(49)	(50)
Hyperplasia	1 (2%)	1 (2%)	
Parathyroid gland	(47)	(46)	(48)
Hyperplasia	4 (9%)	4 (9%)	3 (6%)
Pituitary gland	(49)	(49)	(49)
Pars distalis, angiectasis	6 (12%)	5 (10%)	11 (22%)
Pars distalis, cyst	2 (4%)	1 (2%)	1 (2%)
Pars distalis, hemorrhage, focal	1 (2%)		1 (2%)
Pars distalis, hyperplasia, focal	2 (4%)	5 (10%)	2 (4%)
Pars distalis, pigmentation, hemosiderin	4 (8%)	2 (4%)	7 (14%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
Thyroid gland	(50)	(48)	(50)
Ultimobranchial cyst	1 (2%)	2 (4%)	1 (2%)
C-cell, hyperplasia, focal	1 (2%)	2 (4%)	1 (2%)
C-cell, hyperplasia, multifocal	1 (2%)		1 (2%)
Follicle, cyst	2 (4%)	1 (2%)	1 (2%)
Follicle, hyperplasia, cystic, focal	1 (2%)		
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Preputial gland	(49)	(48)	(43)
Hyperplasia			1 (2%)
Inflammation, chronic		1 (2%)	
Inflammation, subacute	2 (4%)		1 (2%)
Inflammation, suppurative, acute	4 (8%)	8 (17%)	6 (14%)
Duct, cyst	3 (6%)	6 (13%)	3 (7%)
Prostate	(50)	(49)	(50)
Concretion		1 (2%)	
Cyst			1 (2%)
Edema			1 (2%)
Fibrosis		1 (2%)	
Hemorrhage	1 (2%)		
Inflammation, chronic	5 (10%)	1 (2%)	1 (2%)
Inflammation, subacute		3 (6%)	1 (2%)
Inflammation, suppurative, acute	19 (38%)	16 (33%)	19 (38%)
Seminal vesicle		(4)	(2)
Inflammation, chronic		1 (25%)	
Inflammation, suppurative, acute			1 (50%)
Testes	(49)	(50)	(50)
Angiectasis			1 (2%)
Atrophy	2 (4%)	2 (4%)	4 (8%)
Inflammation, suppurative, acute	1 (2%)		
Mineralization		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
Bone marrow	(50)	(50)	(50)
Hyperplasia		1 (2%)	2 (4%)
Lymph node	(50)	(49)	(50)
Deep cervical, congestion		1 (2%)	
Iliac, ectasia	1 (2%)		
Inguinal, hyperplasia	1 (2%)		
Lumbar, ectasia		2 (4%)	
Mandibular, congestion			1 (2%)
Mandibular, ectasia	7 (14%)	7 (14%)	6 (12%)
Mediastinal, congestion			1 (2%)
Mediastinal, hyperplasia	1 (2%)		
Mesenteric, angiectasis	2 (4%)		1 (2%)
Mesenteric, congestion	1 (2%)		
Mesenteric, ectasia	2 (4%)	5 (10%)	4 (8%)
Pancreatic, ectasia		1 (2%)	1 (2%)
Pancreatic, hyperplasia		1 (2%)	

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
Spleen	(50)	(50)	(50)
Atrophy	2 (4%)		1 (2%)
Congestion		1 (2%)	1 (2%)
Developmental malformation	1 (2%)		
Fibrosis	7 (14%)	7 (14%)	7 (14%)
Hematopoietic cell proliferation	1 (2%)	4 (8%)	5 (10%)
Inflammation, suppurative, acute		1 (2%)	
Necrosis, focal		1 (2%)	1 (2%)
Pigmentation, hemosiderin			1 (2%)
Capsule, fibrosis, focal	1 (2%)		
Thymus	(47)	(47)	(47)
Cyst		1 (2%)	1 (2%)
Mediastinum, inflammation, suppurative, acute		1 (2%)	
INTEGUMENTARY SYSTEM			
Mammary gland	(50)	(49)	(46)
Granuloma			1 (2%)
Inflammation, chronic, focal			1 (2%)
Pigmentation, hemosiderin			1 (2%)
Duct, cyst	10 (20%)	18 (37%)	22 (48%)
Skin	(50)	(50)	(50)
Abscess			1 (2%)
Alopecia	1 (2%)		
Cyst epithelial inclusion	1 (2%)	2 (4%)	
Hyperkeratosis, focal	1 (2%)	1 (2%)	1 (2%)
Hyperplasia		1 (2%)	
Hyperplasia, focal	1 (2%)	1 (2%)	2 (4%)
Inflammation, chronic, focal		1 (2%)	1 (2%)
Inflammation, granulomatous, focal	1 (2%)		1 (2%)
Inflammation, subacute, focal		1 (2%)	1 (2%)
Inflammation, suppurative, acute, focal	2 (4%)	2 (4%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
Bone	(50)	(50)	(50)
Fibrous osteodystrophy	3 (6%)	3 (6%)	2 (4%)
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Bacterium			1 (2%)
Compression	1 (2%)	1 (2%)	1 (2%)
Degeneration, multifocal	2 (4%)	4 (8%)	7 (14%)
Hemorrhage, multifocal	2 (4%)	1 (2%)	2 (4%)
Necrosis, focal			1 (2%)
Thrombus			1 (2%)
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Congestion	1 (2%)	3 (6%)	1 (2%)
Cyst	1 (2%)		
Foreign body			1 (2%)
Granuloma			1 (2%)
Hemorrhage, multifocal	2 (4%)	1 (2%)	
Hyperplasia, histiocyte		1 (2%)	
Inflammation, subacute, multifocal		1 (2%)	1 (2%)
Mineralization	1 (2%)	1 (2%)	
Alveolar epithelium, hyperplasia, focal	1 (2%)	2 (4%)	1 (2%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
RESPIRATORY SYSTEM (Continued)			
Nose	(50)	(50)	(45)
Foreign body	3 (6%)	8 (16%)	7 (16%)
Fungus	11 (22%)	20 (40%)	10 (22%)
Hemorrhage			2 (4%)
Inflammation, chronic			1 (2%)
Inflammation, suppurative, acute	14 (28%)	20 (40%)	14 (31%)
Nasolacrimal duct, inflammation, suppurative, acute	3 (6%)	1 (2%)	1 (2%)
Trachea	(50)	(49)	(50)
Inflammation, subacute			1 (2%)
SPECIAL SENSES SYSTEM			
Eye	(4)	(8)	(18)
Cataract	1 (25%)	3 (38%)	13 (72%)
Hemorrhage		1 (13%)	2 (11%)
Synechia	1 (25%)		
Cornea, inflammation, chronic	1 (25%)		
Cornea, inflammation, subacute, diffuse		1 (13%)	
Cornea, inflammation, subacute, focal		1 (13%)	
Retina, degeneration	1 (25%)	6 (75%)	17 (94%)
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Bacterium		1 (2%)	
Fibrosis, focal		1 (2%)	
Hydronephrosis			1 (2%)
Inflammation, chronic, focal		1 (2%)	
Inflammation, suppurative, acute		1 (2%)	1 (2%)
Nephropathy, chronic	49 (98%)	48 (96%)	49 (98%)
Pigmentation, hemosiderin		1 (2%)	
Cortex, cyst	3 (6%)	2 (4%)	2 (4%)
Cortex, mineralization	1 (2%)	2 (4%)	2 (4%)
Cortex, necrosis, focal		1 (2%)	
Papilla, necrosis			1 (2%)
Pelvis, inflammation, suppurative, acute			1 (2%)
Urinary bladder	(50)	(49)	(50)
Hemorrhage			1 (2%)
Inflammation, suppurative, acute			1 (2%)

APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Intestine small	(50)	(49)	(50)
Duodenum, lymphoma malignant histiocytic		1 (2%)	
Peyer's patch, leukemia mononuclear		1 (2%)	
Liver	(50)	(49)	(50)
Leukemia mononuclear	11 (22%)	11 (22%)	9 (18%)
Neoplastic nodule		1 (2%)	
Sarcoma			1 (2%)
Pancreas	(48)	(49)	(50)
Adenoma	1 (2%)		
Leukemia mononuclear			1 (2%)
Lymphoma malignant histiocytic		1 (2%)	
Salivary glands	(50)	(49)	(49)
Leukemia mononuclear		1 (2%)	1 (2%)
Stomach	(50)	(50)	(50)
Leukemia mononuclear		2 (4%)	2 (4%)
Lymphoma malignant histiocytic		1 (2%)	
Tongue	*(50)	*(50)	*(50)
Papilloma squamous			1 (2%)
CARDIOVASCULAR SYSTEM			
Heart	(50)	(50)	(50)
Leukemia mononuclear	2 (4%)	3 (6%)	4 (8%)
Lymphoma malignant histiocytic		1 (2%)	
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(50)	(50)
Leukemia mononuclear	3 (6%)	7 (14%)	5 (10%)
Osteosarcoma, metastatic, bone		1 (2%)	
Sarcoma stromal, metastatic, uterus		1 (2%)	
Cortex, adenoma	1 (2%)	3 (6%)	
Medulla, pheochromocytoma malignant			2 (4%)
Medulla, pheochromocytoma benign	3 (6%)	3 (6%)	7 (14%)
Medulla, pheochromocytoma benign, multiple			1 (2%)
Islets, pancreatic	(48)	(49)	(50)
Adenoma	2 (4%)		
Carcinoma	1 (2%)		1 (2%)
Parathyroid gland	(46)	(48)	(46)
Adenoma		1 (2%)	
Pituitary gland	(49)	(49)	(50)
Leukemia mononuclear	2 (4%)	3 (6%)	3 (6%)
Pars distalis, adenoma	31 (63%)	29 (59%)	28 (56%)
Pars intermedia, adenoma			1 (2%)
Thyroid gland	(50)	(50)	(50)
C-cell, adenoma	4 (8%)	3 (6%)	4 (8%)
C-cell, carcinoma		1 (2%)	2 (4%)
Follicular cell, adenoma	1 (2%)		
Follicular cell, carcinoma			1 (2%)
GENERAL BODY SYSTEM			
None			

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
GENITAL SYSTEM			
Clitoral gland	(42)	(40)	(39)
Adenoma	5 (12%)	4 (10%)	2 (5%)
Carcinoma	1 (2%)	4 (10%)	3 (8%)
Papilloma squamous	1 (2%)		
Sarcoma			1 (3%)
Ovary	(50)	(49)	(50)
Leukemia mononuclear	1 (2%)	2 (4%)	2 (4%)
Sarcoma			1 (2%)
Uterus	(49)	(50)	(50)
Adenocarcinoma	1 (2%)	1 (2%)	
Leiomyoma			1 (2%)
Leukemia mononuclear		2 (4%)	2 (4%)
Polyp stromal	7 (14%)	12 (24%)	13 (26%)
Sarcoma			1 (2%)
Sarcoma stromal	3 (6%)	1 (2%)	
Cervix, leiomyoma		1 (2%)	
HEMATOPOIETIC SYSTEM			
Blood	*(50)	*(50)	*(50)
Leukemia mononuclear	6 (12%)	4 (8%)	5 (10%)
Bone marrow	(50)	(50)	(50)
Leukemia mononuclear	2 (4%)	5 (10%)	4 (8%)
Sarcoma			1 (2%)
Lymph node	(50)	(49)	(50)
Bronchial, leukemia mononuclear		1 (2%)	
Deep cervical, leukemia mononuclear	3 (6%)	1 (2%)	1 (2%)
Inguinal, leukemia mononuclear	1 (2%)		
Mandibular, leukemia mononuclear	3 (6%)	2 (4%)	3 (6%)
Mediastinal, leukemia mononuclear	1 (2%)	1 (2%)	
Mesenteric, leukemia mononuclear	2 (4%)	3 (6%)	4 (8%)
Pancreatic, leukemia mononuclear		2 (4%)	1 (2%)
Renal, leukemia mononuclear		1 (2%)	1 (2%)
Spleen	(49)	(49)	(50)
Leukemia mononuclear	11 (22%)	10 (20%)	10 (20%)
Thymus	(43)	(46)	(49)
Leukemia mononuclear	1 (2%)	1 (2%)	2 (4%)
Lymphoma malignant histiocytic		1 (2%)	
Mediastinum, lymphoma malignant histiocytic		1 (2%)	
INTEGUMENTARY SYSTEM			
Mammary gland	(50)	(50)	(50)
Adenocarcinoma	3 (6%)	1 (2%)	2 (4%)
Adenoma	1 (2%)		
Fibroadenoma	12 (24%)	12 (24%)	11 (22%)
Fibroadenoma, multiple	7 (14%)	3 (6%)	6 (12%)
Sarcoma			1 (2%)
Skin	(50)	(50)	(50)
Basal cell adenoma	1 (2%)		1 (2%)
Keratoacanthoma	1 (2%)	1 (2%)	
Papilloma squamous			1 (2%)
Subcutaneous tissue, fibroma		2 (4%)	
Subcutaneous tissue, lipoma			1 (2%)
Subcutaneous tissue, sarcoma			1 (2%)
Subcutaneous tissue, schwannoma benign			1 (2%)

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
MUSCULOSKELETAL SYSTEM			
Bone	(50)	(50)	(50)
Osteosarcoma	1 (2%)	1 (2%)	
Skeletal muscle	*(50)	*(50)	*(50)
Leukemia mononuclear	1 (2%)		
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Astrocytoma malignant			1 (2%)
Glioma malignant		1 (2%)	
Leukemia mononuclear	1 (2%)	3 (6%)	1 (2%)
Sarcoma			1 (2%)
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Leukemia mononuclear	9 (18%)	9 (18%)	9 (18%)
Lymphoma malignant histiocytic		1 (2%)	
Osteosarcoma, metastatic, bone		1 (2%)	
Sarcoma			2 (4%)
Mediastinum, leukemia mononuclear			1 (2%)
Mediastinum, lymphoma malignant histiocytic		1 (2%)	
Nose	(49)	(50)	(47)
Leukemia mononuclear		1 (2%)	
SPECIAL SENSES SYSTEM			
Zymbal gland	50)	*(50)	*(50)
Carcinoma	1 (2%)		
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Leukemia mononuclear		3 (6%)	5 (10%)
Renal tubule, carcinoma			1 (2%)
Urinary bladder	(50)	(50)	(50)
Leukemia mononuclear	1 (2%)		
Transitional epithelium, papilloma			1 (2%)
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Leukemia mononuclear	11 (22%)	11 (22%)	10 (20%)
Lymphoma malignant histiocytic		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Moribund	17	16	18
Terminal sacrifice	29	30	30
Dead	4	4	2

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary neoplasms **	48	47	49
Total primary neoplasms	100	97	113
Total animals with benign neoplasms	43	41	46
Total benign neoplasms	78	75	80
Total animals with malignant neoplasms	21	22	20
Total malignant neoplasms	22	22	33
Total animals with secondary neoplasms ***		2	
Total secondary neoplasms		3	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: UNTREATED CONTROL

WEEKS ON STUDY	058	076	077	086	086	087	090	093	094	095	095	096	096	097	100	100	100	104	104	104	105	105	105	105	105
CARCASS ID	321	311	341	361	351	401	331	371	391	381	342	382	372	352	373	392	393	353	332	362	322	354	355	374	402
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X	X		X		X				X	X		X			X	
Mesentery																							+		
Pancreas	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X																
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X			X													
Cortex, adenoma																									
Medulla, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X										
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	M	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X																
Pars distalis, adenoma	X	X		X			X				X		X		X	X	X	X	X	X	X		X	X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																						X			
Follicular cell, adenoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Clitoral gland	M	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	M	M	+	+	+	+	+	+
Adenoma												X												X	
Carcinoma										X															
Papilloma squamous																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear												X													
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																				X					
Polyp stromal										X								X			X				
Sarcoma stromal		X	X										X												
Vagina																+									

+: Tissue examined microscopically
: Not examined
−: Present but not examined microscopically
I: Insufficient tissue

M: Missing
A: Autolysis precludes examination
X: Incidence of listed morphology

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	
CARCASS ID	312	313	314	315	323	324	325	333	334	335	343	344	345	363	364	365	375	383	384	385	394	395	403	404	405	TOTAL: TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear								X		X																11
Mesentery									+																	2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Adenoma																						X				1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										2
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										3
Cortex, adenoma												X														1
Medulla, pheochromocytoma benign							X										X					X				3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Adenoma																X										2
Carcinoma										X																1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										2
Pars distalis, adenoma		X	X		X	X			X	X	X		X		X	X		X		X	X	X		X	X	31
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma							X							X	X											4
Follicular cell, adenoma	X																									1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Clitoral gland	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Adenoma						X					X				X											5
Carcinoma																										1
Papilloma squamous							X																			1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49
Adenocarcinoma																										1
Polyp stromal		X										X	X									X				7
Sarcoma stromal																										3
Vagina																										1

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	058	076	077	086	086	087	090	093	094	095	095	096	096	097	100	100	100	104	104	104	105	105	105	105	105
CARCASS ID	321	311	341	361	351	401	331	371	391	381	342	382	372	352	373	392	393	353	332	362	322	354	355	374	402
HEMATOPOIETIC SYSTEM																									
Blood	+	+	+	+						+	+	+		+	+	+	+	+	+	+	+	+	+		+
Leukemia mononuclear												X						X	X		X				
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X																
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deep cervical, leukemia mononuclear								X											X		X				
Inguinal, leukemia mononuclear								X																	
Mandibular, leukemia mononuclear								X											X		X				
Mediastinal, leukemia mononuclear								X																	
Mesenteric, leukemia mononuclear								X											X						
Spleen	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X	X		X		X				X	X		X			X	
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	M	+	M	+	+	+	+	+	M
Leukemia mononuclear								X																	
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																						X			
Adenoma																									
Fibroadenoma					X							X	X					X	X					X	X
Fibroadenoma, multiple																X	X								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma							X																		
Skeletal muscle								+																	
Leukemia mononuclear								X																	
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear									X																
Spinal cord				+																			+		
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X			X		X				X	X		X				
Nose	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Ear																							+		
Eye								+																	
Lacrimal gland																									
Zymbal gland				+																					
Carcinoma				X																					
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X																	

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	TOTAL:
CARCASS ID	312	313	314	315	323	324	325	333	334	335	343	344	345	363	364	365	375	383	384	385	394	395	403	404	405	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Blood	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Leukemia mononuclear								X			X															6
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										2
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Deep cervical, leukemia mononuclear																										3
Inguinal, leukemia mononuclear																										1
Mandibular, leukemia mononuclear																										3
Mediastinal, leukemia mononuclear																										1
Mesenteric, leukemia mononuclear																										2
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear								X			X															11
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	43
Leukemia mononuclear																										1
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma							X								X											3
Adenoma																					X					1
Fibroadenoma							X			X							X					X			X	12
Fibroadenoma, multiple			X					X						X		X			X							7
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma							X																			1
Keratoacanthoma																							X			1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Skeletal muscle																										1
Leukemia mononuclear																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Spinal cord																										2
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear								X			X															9
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Ear																			+	+		+				4
Eye																			+							2
Lacrimal gland															+											1
Zymbal gland																										1
Carcinoma																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: LOW DOSE

WEEKS ON STUDY	0 8 2	0 8 2	0 8 3	0 8 7	0 9 2	0 9 2	0 9 4	0 9 5	0 9 5	0 9 6	0 9 7	1 0 0	1 0 0	1 0 0	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5
CARCASS ID	5 3 1	6 0 1	5 3 2	5 6 1	5 6 2	5 1 1	6 0 2	5 8 1	5 9 1	5 4 1	5 4 2	5 8 2	5 5 1	5 4 3	5 9 2	5 4 4	5 7 1	5 6 3	6 0 3	5 7 2	5 1 2	5 1 3	5 1 4	5 1 5	5 2 1
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Duodenum, lymphoma malignant histiocytic								X																	
Peyer's patch, leukemia mononuclear									X																
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X		X	X			X	X			X		X			X							
Neoplastic nodule														X											
Mesentery				+																					
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic								X																	
Salivary glands	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear									X																
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X				X																
Lymphoma malignant histiocytic								X																	
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X				X						X										
Lymphoma malignant histiocytic								X																	
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X		X				X	X					X			X							
Osteosarcoma, metastatic, bone	X																								
Sarcoma stromal, metastatic, uterus																									
Cortex, adenoma					X			X																	
Medulla, pheochromocytoma benign					X												X								
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Pituitary gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X				X						X										
Pars distalis, adenoma		X			X	X	X	X			X	X	X	X		X	X	X			X	X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																			X						
C-cell, carcinoma																							X		
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Clitoral gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	M	+	M	+	+	+	+	+
Adenoma							X		X																
Carcinoma				X										X								X			
Ovary	+	+	+	+	+	+	+	+	+					+	+	+	+	+	+	+	+	+	M	+	+
Leukemia mononuclear					X				X																
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																							X		
Leukemia mononuclear					X				X																
Polyp stromal						X	X					X						X	X						X
Sarcoma stromal																									
Cervix, leiomyoma					X																				

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	TOTAL:
CARCASS ID	522	523	524	525	533	534	535	545	552	553	554	555	564	565	573	574	575	583	584	585	593	594	595	604	605	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Duodenum, lymphoma malignant histiocytic																										1
Peyer's patch, leukemia mononuclear																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	49
Leukemia mononuclear							X				X								X							11
Neoplastic nodule																										1
Mesentery		+																								2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant histiocytic																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										2
Lymphoma malignant histiocytic																										1
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										3
Lymphoma malignant histiocytic																										1
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																			X							7
Osteosarcoma, metastatic, bone																										1
Sarcoma stromal, metastatic, uterus																					X					1
Cortex, adenoma						X																				3
Medulla, pheochromocytoma benign																		X								3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	48
Adenoma								X																		1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										3
Pars distalis, adenoma				X	X		X	X	X			X	X	X	X	X	X				X	X	X		X	29
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma							X																			3
C-cell, carcinoma													X													1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Clitoral gland	+	+	M	+	+	+	M	+	+	+	+	+	M	+	+	M	+	+	M	M	+	+	+	+	+	40
Adenoma																		X						X		4
Carcinoma					X																					4
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																										1
Leukemia mononuclear																										2
Polyp stromal	X	X						X	X						X					X					12
Sarcoma stromal																					X					1
Cervix, leiomyoma																										1

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

WEEKS ON STUDY	0 8 2	0 8 2	0 8 3	0 8 7	0 9 2	0 9 2	0 9 4	0 9 5	0 9 5	0 9 6	0 9 7	1 0 0	1 0 0	1 0 0	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5
CARCASS ID	5 3 1	6 0 1	5 3 2	5 6 1	5 6 2	5 1 1	6 0 2	5 8 1	5 9 1	5 4 1	5 4 2	5 8 2	5 5 1	5 4 3	5 9 2	5 4 4	5 7 1	5 6 3	6 0 3	5 7 2	5 1 2	5 1 3	5 1 4	5 1 5	5 2 1
HEMATOPOIETIC SYSTEM																									
Blood								+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear									X				X					X							
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X				X	X					X										
Lymph node	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bronchial, leukemia mononuclear			X																						
Deep cervical, leukemia mononuclear					X																				
Mandibular, leukemia mononuclear					X				X																
Mediastinal, leukemia mononuclear									X																
Mesenteric, leukemia mononuclear			X		X				X																
Pancreatic, leukemia mononuclear					X				X																
Renal, leukemia mononuclear									X																
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X		X	X			X	X			X					X							
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Leukemia mononuclear									X																
Lymphoma malignant histiocytic								X																	
Mediastinum, lymphoma malignant histiocytic								X																	
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Fibroadenoma		X																				X			X
Fibroadenoma, multiple														X											
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue, fibroma																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma	X																								
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant																									
Leukemia mononuclear					X				X						X										
Spinal cord																			+						
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X		X	X			X	X					X			X							
Lymphoma malignant histiocytic								X																	
Osteosarcoma, metastatic, bone	X																								
Mediastinum, lymphoma malignant histiocytic								X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X																				
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Ear												+	+							+				+	
Eye										+	+			+							+	+	+	+	+
Lacrimal gland																								+	
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X				X						X										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	106	TOTAL:
CARCASS ID	522	523	524	525	533	534	535	545	552	553	554	555	564	565	573	574	575	583	584	585	593	594	595	604	605	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Blood	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Leukemia mononuclear																			X							4
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear							X																			5
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Bronchial, leukemia mononuclear																										1
Deep cervical, leukemia mononuclear																										1
Mandibular, leukemia mononuclear																										2
Mediastinal, leukemia mononuclear																										1
Mesenteric, leukemia mononuclear																										3
Pancreatic, leukemia mononuclear																										2
Renal, leukemia mononuclear																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear							X				X								X							10
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	46
Leukemia mononuclear																										1
Lymphoma malignant histiocytic																										1
Mediastinum, lymphoma malignant histiocytic																										1
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																	X									1
Fibroadenoma		X		X	X				X		X	X				X			X						X	12
Fibroadenoma, multiple							X						X													3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																			X							1
Subcutaneous tissue, fibroma																		X						X		2
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma malignant		X																								1
Leukemia mononuclear																										3
Spinal cord																										1
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear							X												X							9
Lymphoma malignant histiocytic																										1
Osteosarcoma, metastatic, bone																										1
Mediastinum, lymphoma malignant histiocytic																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Ear		+	+			+	+	+	+											+						11
Eye	+	+	+	+	+	+	+	+	+	+	+	+							+							21
Lacrimal gland			+			+																				3
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: HIGH DOSE

WEEKS ON STUDY	052	058	060	076	079	083	089	089	090	091	091	092	095	095	100	100	104	104	105	105	105	105	105	105	105
CARCASS ID	481	451	501	452	502	453	441	442	411	421	491	471	472	431	412	461	432	482	462	492	413	414	415	422	423
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	M	+	+	+	+	+	+	+	+	M
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X				X							X				X		X						
Sarcoma			X																						
Mesentery		+			+																		+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Tongue																			+						
Papilloma squamous																			X						
Tooth																						+			
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X											X												
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X							X				X								
Medulla, pheochromocytoma malignant																						X			
Medulla, pheochromocytoma benign				X													X			X				X	
Medulla, pheochromocytoma benign, multiple																		X							
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X					X																			
Pars distalis, adenoma		X			X	X	X	X	X	X		X			X	X	X	X				X	X	X	X
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
C-cell, carcinoma				X									X												
Follicular cell, carcinoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Clitoral gland	M	M	M	M	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X							X										
Carcinoma											X														
Sarcoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Sarcoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									
Leukemia mononuclear	X																								
Polyp stromal						X					X			X	X				X			X		X	
Sarcoma																									

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	424	425	433	434	435	443	444	445	454	455	463	464	465	473	474	475	483	484	485	503	493	494	495	504	505	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X					X					X					9
Sarcoma																										1
Mesentery													+		+						+	+		+	+	9
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	49
Leukemia mononuclear																										1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																				X						2
Tongue																										1
Papilloma squamous																										1
Tooth																										1
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X																4
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear															X					X						5
Medulla, pheochromocytoma *malignant*																		X								2
Medulla, pheochromocytoma benign				X	X																X					7
Medulla, pheochromocytoma benign, multiple,																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																									X	1
Parathyroid gland	+	M	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																				X						3
Pars distalis, adenoma	X	X			X		X		X		X	X	X	X	X	X									X	28
Pars intermedia, adenoma																		X								1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma											X				X		X							X		4
C-cell, carcinoma																										2
Follicular cell, carcinoma																								X		1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Clitoral gland	+	M	+	M	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	39
Adenoma																										2
Carcinoma																X									X	3
Sarcoma																		X								1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																				X						2
Sarcoma																		X								1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma																									X	1
Leukemia mononuclear																				X						2
Polyp stromal	X									X				X		X		X					X			13
Sarcoma																		X								1

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

WEEKS ON STUDY	052	058	060	076	079	083	089	089	090	091	091	092	095	095	100	100	104	104	105	105	105	105	105	105
CARCASS ID	481	451	501	452	502	453	441	442	411	421	491	471	472	431	412	461	432	482	462	492	413	414	415	422	423
HEMATOPOIETIC SYSTEM																									
Blood	+	+	+	+	+	+	+		+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X											X						X						
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X												X												
Sarcoma			X																						
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deep cervical, leukemia mononuclear													X												
Mandibular, leukemia mononuclear	X												X												
Mesenteric, leukemia mononuclear						X							X				X								
Pancreatic, leukemia mononuclear																									
Renal, leukemia mononuclear																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X				X							X				X		X						
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X												X												
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Fibroadenoma										X		X		X	X	X									
Fibroadenoma, multiple									X												X	X			
Sarcoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																						X			
Papilloma squamous																									
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, sarcoma																									
Subcutaneous tissue, schwannoma benign																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																			X						
Leukemia mononuclear						X																			
Sarcoma			X																						
Spinal cord			+								+		+												
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X				X							X				X		X						
Sarcoma		X	X																						
Mediastinum, leukemia mononuclear	X																								
Nose	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Ear			+						+				+				+			+	+		+	+	
Eye			+											+			+				+		+		
Lacrimal gland																									
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X					X							X				X								
Renal tubule, carcinoma				X																					
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma								X																	

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	424	425	433	434	435	443	444	445	454	455	463	464	465	473	474	475	483	484	485	503	493	494	495	504	505	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Blood	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leukemia mononuclear										X																5
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X										X						4
Sarcoma																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Deep cervical, leukemia mononuclear																										1
Mandibular, leukemia mononuclear																				X						3
Mesenteric, leukemia mononuclear																				X						4
Pancreatic, leukemia mononuclear																				X						1
Renal, leukemia mononuclear																				X						1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X					X					X					X							10
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										2
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma		X										X														2
Fibroadenoma	X	X					X	X	X														X			11
Fibroadenoma, multiple			X													X			X							6
Sarcoma																		X								1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma																										1
Papilloma squamous						X																				1
Subcutaneous tissue, lipoma																			X							1
Subcutaneous tissue, sarcoma																		X								1
Subcutan. tissue, schwannoma benign																				X						1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Leukemia mononuclear																										1
Sarcoma																										1
Spinal cord																										3
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear										X					X					X							9
Sarcoma																										2
Mediastinum, leukemia mononuclear																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Ear					+					+					+	+										12
Eye										+																6
Lacrimal gland																				+						1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																				X						5
Renal tubule, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma																										1

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Control	120 ppm	250 ppm
Adrenal Cortex: Adenoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	3.4%	7.6%	0.0%
Terminal Rates (c)	1/29 (3%)	1/30 (3%)	0/30 (0%)
Day of First Observation	734	638	
Life Table Tests (d)	P=0.376N	P=0.324	P=0.493N
Logistic Regression Tests (d)	P=0.363N	P=0.289	P=0.493N
Cochran-Armitage Trend Test (d)	P=0.367N		
Fisher Exact Test (d)		P=0.309	P=0.500N
Adrenal Medulla: Pheochromocytoma			
Overall Rates (a)	3/50 (6%)	3/50 (6%)	8/50 (16%)
Adjusted Rates (b)	10.3%	8.1%	22.7%
Terminal Rates (c)	3/29 (10%)	1/30 (3%)	4/30 (13%)
Day of First Observation	734	638	531
Life Table Tests (d)	P=0.070	P=0.639N	P=0.120
Logistic Regression Tests (d)	P=0.053	P=0.644N	P=0.092
Cochran-Armitage Trend Test (d)	P=0.059		
Fisher Exact Test (d)		P=0.661N	P=0.100
Adrenal Medulla: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	3/50 (6%)	3/50 (6%)	10/50 (20%)
Adjusted Rates (b)	10.3%	8.1%	28.6%
Terminal Rates (c)	3/29 (10%)	1/30 (3%)	6/30 (20%)
Day of First Observation	734	638	531
Life Table Tests (d)	P=0.021	P=0.639N	P=0.047
Logistic Regression Tests (d)	P=0.014	P=0.644N	P=0.032
Cochran-Armitage Trend Test (d)	P=0.017		
Fisher Exact Test (d)		P=0.661N	P=0.036
Clitoral Gland: Adenoma			
Overall Rates (a)	5/42 (12%)	4/40 (10%)	2/39 (5%)
Adjusted Rates (b)	17.0%	12.5%	5.0%
Terminal Rates (c)	4/27 (15%)	2/24 (8%)	0/25 (0%)
Day of First Observation	667	657	623
Life Table Tests (d)	P=0.199N	P=0.537N	P=0.245N
Logistic Regression Tests (d)	P=0.196N	P=0.535N	P=0.247N
Cochran-Armitage Trend Test (d)	P=0.195N		
Fisher Exact Test (d)		P=0.532N	P=0.248N
Clitoral Gland: Adenoma or Squamous Papilloma			
Overall Rates (a)	6/42 (14%)	4/40 (10%)	2/39 (5%)
Adjusted Rates (b)	20.6%	12.5%	5.0%
Terminal Rates (c)	5/27 (19%)	2/24 (8%)	0/25 (0%)
Day of First Observation	667	657	623
Life Table Tests (d)	P=0.123N	P=0.412N	P=0.159N
Logistic Regression Tests (d)	P=0.119N	P=0.405N	P=0.157N
Cochran-Armitage Trend Test (d)	P=0.118N		
Fisher Exact Test (d)		P=0.401N	P=0.157N
Clitoral Gland: Carcinoma			
Overall Rates (a)	1/42 (2%)	4/40 (10%)	3/39 (8%)
Adjusted Rates (b)	2.4%	12.7%	10.3%
Terminal Rates (c)	0/27 (0%)	2/24 (8%)	2/25 (8%)
Day of First Observation	661	603	635
Life Table Tests (d)	P=0.242	P=0.179	P=0.279
Logistic Regression Tests (d)	P=0.229	P=0.167	P=0.273
Cochran-Armitage Trend Test (d)	P=0.234		
Fisher Exact Test (d)		P=0.165	P=0.280

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Control	120 ppm	250 ppm
Clitoral Gland: Adenoma or Carcinoma			
Overall Rates (a)	6/42 (14%)	8/40 (20%)	5/39 (13%)
Adjusted Rates (b)	19.0%	24.3%	14.8%
Terminal Rates (c)	4/27 (15%)	4/24 (17%)	2/25 (8%)
Day of First Observation	661	603	623
Life Table Tests (d)	P=0.485N	P=0.357	P=0.546N
Logistic Regression Tests (d)	P=0.496N	P=0.349	P=0.558N
Cochran-Armitage Trend Test (d)	P=0.491N		
Fisher Exact Test (d)		P=0.347	P=0.553N
Clitoral Gland: Adenoma, Squamous Papilloma, or Carcinoma			
Overall Rates (a)	7/42 (17%)	8/40 (20%)	5/39 (13%)
Adjusted Rates (b)	22.5%	24.3%	14.8%
Terminal Rates (c)	5/27 (19%)	4/24 (17%)	2/25 (8%)
Day of First Observation	661	603	623
Life Table Tests (d)	P=0.378N	P=0.460	P=0.430N
Logistic Regression Tests (d)	P=0.382N	P=0.459	P=0.434N
Cochran-Armitage Trend Test (d)	P=0.378N		
Fisher Exact Test (d)		P=0.458	P=0.432N
Pancreatic Islets: Adenoma or Carcinoma			
Overall Rates (a)	3/48 (6%)	0/49 (0%)	1/50 (2%)
Adjusted Rates (b)	9.7%	0.0%	3.3%
Terminal Rates (c)	2/28 (7%)	0/30 (0%)	1/30 (3%)
Day of First Observation	695		734
Life Table Tests (d)	P=0.178N	P=0.110N	P=0.290N
Logistic Regression Tests (d)	P=0.181N	P=0.106N	P=0.298N
Cochran-Armitage Trend Test (d)	P=0.179N		
Fisher Exact Test (d)		P=0.117N	P=0.293N
Mammary Gland: Adenocarcinoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted Rates (b)	10.3%	3.3%	6.7%
Terminal Rates (c)	3/29 (10%)	1/30 (3%)	2/30 (7%)
Day of First Observation	734	734	734
Life Table Tests (d)	P=0.397N	P=0.292N	P=0.484N
Logistic Regression Tests (d)	P=0.397N	P=0.292N	P=0.484N
Cochran-Armitage Trend Test (d)	P=0.411N		
Fisher Exact Test (d)		P=0.309N	P=0.500N
Mammary Gland: Fibroadenoma			
Overall Rates (a)	19/50 (38%)	15/50 (30%)	17/50 (34%)
Adjusted Rates (b)	51.8%	46.0%	46.0%
Terminal Rates (c)	12/29 (41%)	13/30 (43%)	11/30 (37%)
Day of First Observation	602	572	629
Life Table Tests (d)	P=0.367N	P=0.223N	P=0.407N
Logistic Regression Tests (d)	P=0.421N	P=0.207N	P=0.457N
Cochran-Armitage Trend Test (d)	P=0.384N		
Fisher Exact Test (d)		P=0.263N	P=0.418N
Mammary Gland: Adenoma or Fibroadenoma			
Overall Rates (a)	20/50 (40%)	15/50 (30%)	17/50 (34%)
Adjusted Rates (b)	54.6%	46.0%	46.0%
Terminal Rates (c)	13/29 (45%)	13/30 (43%)	11/30 (37%)
Day of First Observation	602	572	629
Life Table Tests (d)	P=0.294N	P=0.166N	P=0.335N
Logistic Regression Tests (d)	P=0.342N	P=0.150N	P=0.376N
Cochran-Armitage Trend Test (d)	P=0.308N		
Fisher Exact Test (d)		P=0.201N	P=0.339N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Control	120 ppm	250 ppm
Mammary Gland: Adenoma, Fibroadenoma, or Adenocarcinoma			
Overall Rates (a)	23/50 (46%)	16/50 (32%)	18/50 (36%)
Adjusted Rates (b)	63.1%	49.2%	48.8%
Terminal Rates (c)	16/29 (55%)	14/30 (47%)	12/30 (40%)
Day of First Observation	602	572	629
Life Table Tests (d)	P=0.172N	P=0.083N	P=0.208N
Logistic Regression Tests (d)	P=0.209N	P=0.069N	P=0.237N
Cochran-Armitage Trend Test (d)	P=0.185N		
Fisher Exact Test (d)		P=0.109N	P=0.208N
Pituitary Gland/Pars Distalis: Adenoma			
Overall Rates (a)	31/49 (63%)	29/49 (59%)	28/50 (56%)
Adjusted Rates (b)	73.4%	68.1%	65.6%
Terminal Rates (c)	18/29 (62%)	17/30 (57%)	16/30 (53%)
Day of First Observation	405	572	405
Life Table Tests (d)	P=0.320N	P=0.352N	P=0.346N
Logistic Regression Tests (d)	P=0.273N	P=0.413N	P=0.308N
Cochran-Armitage Trend Test (d)	P=0.265N		
Fisher Exact Test (d)		P=0.418N	P=0.298N
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted Rates (b)	13.8%	9.5%	13.3%
Terminal Rates (c)	4/29 (14%)	2/30 (7%)	4/30 (13%)
Day of First Observation	734	728	734
Life Table Tests (d)	P=0.563N	P=0.477N	P=0.628N
Logistic Regression Tests (d)	P=0.568	P=0.484N	P=0.628N
Cochran-Armitage Trend Test (d)	P=0.575		
Fisher Exact Test (d)		P=0.500N	P=0.643N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	4/50 (8%)	6/50 (12%)
Adjusted Rates (b)	13.8%	12.7%	17.4%
Terminal Rates (c)	4/29 (14%)	3/30 (10%)	4/30 (13%)
Day of First Observation	734	728	531
Life Table Tests (d)	P=0.316	P=0.621N	P=0.379
Logistic Regression Tests (d)	P=0.286	P=0.628N	P=0.356
Cochran-Armitage Trend Test (d)	P=0.302		
Fisher Exact Test (d)		P=0.643N	P=0.370
Uterus: Stromal Polyp			
Overall Rates (a)	7/49 (14%)	12/50 (24%)	13/50 (26%)
Adjusted Rates (b)	21.6%	32.9%	35.9%
Terminal Rates (c)	4/28 (14%)	7/30 (23%)	8/30 (27%)
Day of First Observation	661	643	575
Life Table Tests (d)	P=0.120	P=0.209	P=0.134
Logistic Regression Tests (d)	P=0.087	P=0.195	P=0.102
Cochran-Armitage Trend Test (d)	P=0.101		
Fisher Exact Test (d)		P=0.166	P=0.115
Uterus: Stromal Sarcoma			
Overall Rates (a)	3/49 (6%)	1/50 (2%)	0/50 (0%)
Adjusted Rates (b)	6.6%	3.3%	0.0%
Terminal Rates (c)	0/28 (0%)	1/30 (3%)	0/30 (0%)
Day of First Observation	531	734	
Life Table Tests (d)	P=0.066N	P=0.293N	P=0.132N
Logistic Regression Tests (d)	P=0.036N	P=0.452N	P=0.060N
Cochran-Armitage Trend Test (d)	P=0.061N		
Fisher Exact Test (d)		P=0.301N	P=0.117N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Control	120 ppm	250 ppm
Uterus: Sarcoma or Stromal Sarcoma			
Overall Rates (a)	3/49 (6%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	6.6%	3.3%	3.3%
Terminal Rates (c)	0/28 (0%)	1/30 (3%)	1/30 (3%)
Day of First Observation	531	734	734
Life Table Tests (d)	P=0.211N	P=0.293N	P=0.315N
Logistic Regression Tests (d)	P=0.158N	P=0.452N	P=0.230N
Cochran-Armitage Trend Test (d)	P=0.203N		
Fisher Exact Test (d)		P=0.301N	P=0.301N
Hematopoietic System: Mononuclear Leukemia			
Overall Rates (a)	11/50 (22%)	11/50 (22%)	10/50 (20%)
Adjusted Rates (b)	28.2%	26.3%	25.5%
Terminal Rates (c)	3/29 (10%)	3/30 (10%)	4/30 (13%)
Day of First Observation	646	575	361
Life Table Tests (d)	P=0.461N	P=0.536N	P=0.500N
Logistic Regression Tests (d)	P=0.395N	P=0.557	P=0.458N
Cochran-Armitage Trend Test (d)	P=0.452N		
Fisher Exact Test (d)		P=0.595N	P=0.500N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors 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. A negative trend or lower incidence in a dosed group is indicated by (N).

TABLE B4a. HISTORICAL INCIDENCE OF ADRENAL GLAND MEDULLARY TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls	
	Pheochromocytoma	Pheochromocytoma or Malignant Pheochromocytoma
Historical Incidence at Southern Research Institute		
HC Blue No. 2	3/49	3/49
C.I. Disperse Blue 1	5/48	5/48
Eugenol	1/40	2/40
Stannous chloride	1/50	1/50
D-Mannitol	2/49	2/49
Ziram	1/50	1/50
Propyl gallate	4/50	4/50
Zearalenone	0/50	0/50
HC Blue No. 1	8/50	8/50
TOTAL	25/436 (5.7%)	26/436 (6.0%)
SD (b)	5.08%	4.95%
Range (c)		
High	8/50	8/50
Low	0/50	0/50
Overall Historical Incidence		
TOTAL	92/1,968 (4.7%)	99/1,968 (5.0%)
SD (b)	3.75%	3.70%
Range (c)		
High	8/50	8/50
Low	0/50	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE B4b. HISTORICAL INCIDENCE OF LUNG SARCOMAS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

	Incidence in Controls
Historical Incidence at Southern Research Institute	0/436
Overall Historical Incidence	0/1,974

(a) Data as of April 29, 1987, for studies of at least 104 weeks

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Intestine large	(50)	(49)	(50)
Cecum, parasite metazoan	2 (4%)		
Colon, parasite metazoan	2 (4%)	2 (4%)	
Rectum, parasite metazoan	1 (2%)	1 (2%)	
Liver	(50)	(49)	(50)
Angiectasis, focal	1 (2%)	1 (2%)	1 (2%)
Basophilic focus	11 (22%)	8 (16%)	5 (10%)
Basophilic focus, multiple	18 (36%)	22 (45%)	28 (56%)
Clear cell focus	2 (4%)		
Congestion, diffuse			1 (2%)
Developmental malformation	4 (8%)	4 (8%)	3 (6%)
Granuloma	1 (2%)	1 (2%)	1 (2%)
Granuloma, multifocal	17 (34%)	18 (37%)	16 (32%)
Hematopoietic cell proliferation, multifocal	1 (2%)		2 (4%)
Hemorrhage, multifocal			1 (2%)
Hepatodiaphragmatic nodule		2 (4%)	3 (6%)
Mixed cell focus			1 (2%)
Necrosis, focal	1 (2%)	1 (2%)	1 (2%)
Necrosis, multifocal	2 (4%)		2 (4%)
Pigmentation, hemosiderin, multifocal			1 (2%)
Thrombus, multiple			1 (2%)
Vacuolization cytoplasmic, diffuse	6 (12%)	8 (16%)	7 (14%)
Vacuolization cytoplasmic, focal		2 (4%)	1 (2%)
Biliary tract, hyperplasia	14 (28%)	19 (39%)	13 (26%)
Centrilobular, necrosis	7 (14%)	6 (12%)	7 (14%)
Hepatocyte, hypertrophy	5 (10%)	6 (12%)	4 (8%)
Mesentery	(2)	(2)	(9)
Inflammation, chronic, focal	1 (50%)		
Fat, necrosis, focal	1 (50%)	2 (100%)	9 (100%)
Pancreas	(48)	(49)	(50)
Atrophy	7 (15%)	9 (18%)	8 (16%)
Inflammation, subacute, focal	1 (2%)		
Artery, inflammation, subacute		1 (2%)	
Salivary glands	(50)	(49)	(49)
Inflammation, subacute, multifocal			1 (2%)
Duct, cyst		1 (2%)	2 (4%)
Stomach	(50)	(50)	(50)
Artery, inflammation, subacute	1 (2%)		
Forestomach, edema		1 (2%)	4 (8%)
Forestomach, hyperkeratosis	3 (6%)	5 (10%)	2 (4%)
Forestomach, hyperplasia	3 (6%)	5 (10%)	3 (6%)
Forestomach, inflammation, subacute	4 (8%)	4 (8%)	
Forestomach, perforation	2 (4%)		
Forestomach, ulcer	2 (4%)	4 (8%)	4 (8%)
Forestomach, ulcer, multiple		1 (2%)	1 (2%)
Glandular, edema		1 (2%)	
Glandular, erosion		1 (2%)	1 (2%)
Glandular, inflammation, subacute	2 (4%)	1 (2%)	
Glandular, mineralization	1 (2%)		
Glandular, ulcer		1 (2%)	1 (2%)
Tooth			(1)
Inflammation, suppurative, acute			1 (100%)

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
CARDIOVASCULAR SYSTEM			
Heart	(50)	(50)	(50)
Fibrosis, multifocal	16 (32%)	26 (52%)	22 (44%)
Atrium, mineralization, focal			1 (2%)
Atrium, pigmentation, hemosiderin			1 (2%)
Atrium, thrombus			1 (2%)
ENDOCRINE SYSTEM			
Adrenal gland	(50)	(50)	(50)
Accessory adrenal cortical nodule		1 (2%)	
Cortex, angiectasis, focal	1 (2%)	1 (2%)	
Cortex, angiectasis, multifocal	1 (2%)		1 (2%)
Cortex, congestion, diffuse		1 (2%)	1 (2%)
Cortex, degeneration, fatty, focal	5 (10%)	13 (26%)	7 (14%)
Cortex, degeneration, fatty, multifocal	2 (4%)	4 (8%)	5 (10%)
Cortex, hyperplasia, focal	1 (2%)	2 (4%)	2 (4%)
Cortex, hypertrophy, focal	1 (2%)		
Cortex, necrosis, focal		1 (2%)	
Medulla, hyperplasia, focal	4 (8%)	6 (12%)	8 (16%)
Parathyroid gland	(46)	(48)	(46)
Hyperplasia	1 (2%)		
Pituitary gland	(49)	(49)	(50)
Hemorrhage, focal	1 (2%)	1 (2%)	
Pars distalis, angiectasis	34 (69%)	30 (61%)	30 (60%)
Pars distalis, cyst	11 (22%)	18 (37%)	8 (16%)
Pars distalis, hyperplasia, focal	7 (14%)	7 (14%)	9 (18%)
Pars distalis, pigmentation, hemosiderin	25 (51%)	17 (35%)	15 (30%)
Thyroid gland	(50)	(50)	(50)
C-cell, hyperplasia, focal	3 (6%)	1 (2%)	5 (10%)
C-cell, hyperplasia, multifocal	1 (2%)	2 (4%)	1 (2%)
Follicle, cyst	1 (2%)		
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Clitoral gland	(42)	(40)	(39)
Cyst	5 (12%)	2 (5%)	4 (10%)
Inflammation, subacute, focal	3 (7%)	1 (3%)	1 (3%)
Inflammation, suppurative, acute	1 (2%)	4 (10%)	3 (8%)
Ovary	(50)	(49)	(50)
Cyst	2 (4%)	5 (10%)	6 (12%)
Uterus	(49)	(50)	(50)
Hemorrhage, focal	1 (2%)		1 (2%)
Hydrometria		2 (4%)	1 (2%)
Inflammation, suppurative, acute	2 (4%)		
Necrosis	2 (4%)		
Cervix, abscess		5 (10%)	5 (10%)
Cervix, cyst	2 (4%)	5 (10%)	3 (6%)
Cervix, necrosis		1 (2%)	
Endometrium, hyperplasia, cystic			1 (2%)
Mucosa, cyst	1 (2%)		1 (2%)
Vagina	(1)		
Abscess	1 (100%)		

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM			
Bone marrow	(50)	(50)	(50)
Hyperplasia	3 (6%)		2 (4%)
Lymph node	(50)	(49)	(50)
Deep cervical, congestion		1 (2%)	
Inguinal, hyperplasia		1 (2%)	1 (2%)
Mandibular, ectasia		2 (4%)	1 (2%)
Mandibular, hyperplasia	1 (2%)		
Mesenteric, congestion			1 (2%)
Spleen	(49)	(49)	(50)
Congestion			1 (2%)
Fibrosis, focal	1 (2%)		
Hematopoietic cell proliferation	7 (14%)	5 (10%)	5 (10%)
Hyperplasia, lymphoid, focal		1 (2%)	
Necrosis, focal		1 (2%)	
Thymus	(43)	(46)	(49)
Cyst			1 (2%)
INTEGUMENTARY SYSTEM			
Mammary gland	(50)	(50)	(50)
Hemorrhage	1 (2%)	1 (2%)	
Hyperplasia, glandular	1 (2%)	3 (6%)	1 (2%)
Duct, cyst	47 (94%)	44 (88%)	42 (84%)
Duct, cyst, multiple			1 (2%)
Skin	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)
Foreign body		3 (6%)	
Hyperkeratosis, focal		1 (2%)	
Hyperplasia	1 (2%)		
Hyperplasia, focal		1 (2%)	
Inflammation, granulomatous, multifocal		1 (2%)	
Inflammation, subacute, focal	1 (2%)		
Inflammation, suppurative, acute, focal	1 (2%)	2 (4%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
Bone	(50)	(50)	(50)
Cranium, hyperostosis, focal		1 (2%)	
NERVOUS SYSTEM			
Brain	(50)	(50)	(50)
Compression	4 (8%)	4 (8%)	3 (6%)
Degeneration, multifocal	2 (4%)	7 (14%)	
Hemorrhage, multifocal	1 (2%)	2 (4%)	
Mineralization, focal		1 (2%)	
Necrosis, multifocal			1 (2%)
Cerebellum, mineralization, multifocal		1 (2%)	
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Atelectasis, multifocal		1 (2%)	
Congestion		1 (2%)	
Foreign body, multiple	1 (2%)		
Granuloma, multifocal	1 (2%)		
Hemorrhage, multifocal		2 (4%)	
Hyperplasia, macrophage			1 (2%)
Metaplasia, osseous, multifocal	1 (2%)		
Pigmentation, hemosiderin	1 (2%)		1 (2%)
Alveolar epithelium, hyperplasia, focal			1 (2%)

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
RESPIRATORY SYSTEM (Continued)			
Nose	(49)	(50)	(47)
Foreign body	1 (2%)		
Fungus		2 (4%)	1 (2%)
Inflammation, granulomatous	1 (2%)		
Inflammation, suppurative, acute	2 (4%)	3 (6%)	2 (4%)
Nasolacrimal duct, foreign body			1 (2%)
Nasolacrimal duct, inflammation, suppurative, acute	3 (6%)	1 (2%)	2 (4%)
SPECIAL SENSES SYSTEM			
Ear	(4)	(11)	(12)
Inflammation, suppurative, acute			1 (8%)
Eye	(2)	(21)	(6)
Cataract	1 (50%)	21 (100%)	3 (50%)
Hemorrhage	1 (50%)		
Cornea, inflammation, chronic			2 (33%)
Retina, degeneration	2 (100%)	21 (100%)	5 (83%)
Lacrimal gland	(1)	(3)	(1)
Ectopic harderian	1 (100%)	3 (100%)	1 (100%)
URINARY SYSTEM			
Kidney	(50)	(50)	(50)
Nephropathy, chronic	39 (78%)	37 (74%)	36 (72%)
Cortex, cyst	1 (2%)		
Cortex, inflammation, suppurative, acute, multifocal	1 (2%)		
Medulla, mineralization, focal			1 (2%)
Right, hydronephrosis		1 (2%)	

APPENDIX C

SUMMARY OF LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Gallbladder	(31)	(30)	(38)
Lymphoma malignant histiocytic		1 (3%)	
Lymphoma malignant mixed			1 (3%)
Intestine small	(46)	(46)	(45)
Ileum, Peyer's patch, lymphoma malignant mixed			1 (2%)
Peyer's patch, lymphoma malignant lymphocytic	1 (2%)		
Peyer's patch, lymphoma malignant mixed		1 (2%)	
Liver	(49)	(49)	(50)
Hepatocellular carcinoma	9 (18%)	6 (12%)	5 (10%)
Hepatocellular carcinoma, multiple	1 (2%)		1 (2%)
Hepatocellular adenoma	5 (10%)	7 (14%)	5 (10%)
Lymphoma malignant histiocytic		2 (4%)	1 (2%)
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed			1 (2%)
Mesentery	*(50)	*(50)	*(50)
Lymphoma malignant histiocytic		1 (2%)	
Lymphoma malignant lymphocytic	1 (2%)		
Pancreas	(48)	(49)	(48)
Lymphoma malignant histiocytic		1 (2%)	
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed			1 (2%)
Stomach	(48)	(49)	(50)
Lymphoma malignant histiocytic		1 (2%)	
Lymphoma malignant lymphocytic	1 (2%)		
Forestomach, papilloma squamous	2 (4%)	1 (2%)	1 (2%)
CARDIOVASCULAR SYSTEM			
None			
ENDOCRINE SYSTEM			
Adrenal gland	(49)	(48)	(49)
Lymphoma malignant histiocytic		1 (2%)	
Pheochromocytoma benign			1 (2%)
Cortex, adenoma		1 (2%)	
Thyroid gland	(50)	(49)	(50)
C-cell, carcinoma	1 (2%)		
Follicular cell, adenoma		3 (6%)	3 (6%)
Follicular cell, carcinoma		1 (2%)	
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Preputial gland	*(50)	*(50)	*(50)
Adenoma			1 (2%)
Prostate	(47)	(48)	(50)
Lymphoma malignant mixed			1 (2%)

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM			
Bone marrow	(49)	(49)	(50)
Lymphoma malignant mixed			1 (2%)
Lymph node	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin	2 (4%)	1 (2%)	
Sarcoma, metastatic, skin	1 (2%)		
Bronchial, lymphoma malignant lymphocytic	1 (2%)		
Inguinal, lymphoma malignant histiocytic		3 (6%)	
Inguinal, lymphoma malignant lymphocytic	1 (2%)		
Inguinal, lymphoma malignant mixed			1 (2%)
Mandibular, lymphoma malignant histiocytic		2 (4%)	
Mandibular, lymphoma malignant lymphocytic	1 (2%)		
Mandibular, lymphoma malignant mixed			1 (2%)
Mediastinal, lymphoma malignant histiocytic		1 (2%)	
Mediastinal, lymphoma malignant lymphocytic	2 (4%)		
Mediastinal, lymphoma malignant mixed			1 (2%)
Mesenteric, lymphoma malignant histiocytic		2 (4%)	1 (2%)
Mesenteric, lymphoma malignant lymphocytic	2 (4%)		
Mesenteric, lymphoma malignant	1 (2%)		
Mesenteric, lymphoma malignant mixed	1 (2%)		1 (2%)
Pancreatic, lymphoma malignant histiocytic		1 (2%)	
Pancreatic, lymphoma malignant mixed	1 (2%)		1 (2%)
Renal, lymphoma malignant histiocytic		2 (4%)	
Spleen	(49)	(49)	(48)
Hemangiosarcoma	1 (2%)		
Lymphoma malignant histiocytic		3 (6%)	1 (2%)
Lymphoma malignant lymphocytic	2 (4%)		1 (2%)
Lymphoma malignant mixed	1 (2%)		1 (2%)
Thymus	(31)	(40)	(47)
Lymphoma malignant histiocytic		2 (5%)	
Lymphoma malignant lymphocytic	1 (3%)		
INTEGUMENTARY SYSTEM			
Skin	(48)	(50)	(50)
Papilloma squamous	1 (2%)		
Subcutaneous tissue, fibroma		4 (8%)	2 (4%)
Subcutaneous tissue, fibrosarcoma	9 (19%)	12 (24%)	5 (10%)
Subcutaneous tissue, fibrosarcoma, multiple	1 (2%)		1 (2%)
Subcutaneous tissue, hemangioma	1 (2%)		
Subcutaneous tissue, sarcoma	4 (8%)	2 (4%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
Skeletal muscle	*(50)	*(50)	*(50)
Fibrosarcoma	1 (2%)		
NERVOUS SYSTEM			
None			
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	5 (10%)	4 (8%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)		
Alveolar/bronchiolar carcinoma	3 (6%)	2 (4%)	1 (2%)
Carcinoma, metastatic, thyroid gland	1 (2%)		
Fibrosarcoma, metastatic, skin	2 (4%)	2 (4%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	2 (4%)	2 (4%)	
Lymphoma malignant histiocytic		2 (4%)	1 (2%)
Lymphoma malignant lymphocytic	1 (2%)		

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
SPECIAL SENSES SYSTEM			
Harderian gland	*(50)	*(50)	*(50)
Adenoma	7 (14%)	2 (4%)	2 (4%)
Lymphoma malignant mixed			1 (2%)
URINARY SYSTEM			
Kidney	(50)	(49)	(50)
Fibrosarcoma, metastatic, skin	1 (2%)		
Lymphoma malignant histiocytic		1 (2%)	
Lymphoma malignant mixed			1 (2%)
Cortex, renal tubule, adenoma	1 (2%)		
Urethra	*(50)	*(50)	*(50)
Bulbourethral gland, leiomyosarcoma			1 (2%)
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Hemangiosarcoma	1 (2%)		
Lymphoma malignant lymphocytic	3 (6%)		1 (2%)
Hemangioma	1 (2%)		
Lymphoma malignant	1 (2%)		
Lymphoma malignant mixed	1 (2%)	1 (2%)	1 (2%)
Lymphoma malignant histiocytic		3 (6%)	1 (2%)
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Terminal sacrifice	36	32	38
Dead	5	9	8
Moribund	8	8	4
Moribund sacrifice	1		
Accident		1	
TUMOR SUMMARY			
Total animals with primary neoplasms **	39	33	29
Total primary neoplasms	58	51	37
Total animals with benign neoplasms	18	17	15
Total benign neoplasms	23	23	19
Total animals with malignant neoplasms	30	25	16
Total malignant neoplasms	35	28	18
Total animals with secondary neoplasms ***	8	4	1
Total secondary neoplasms	9	5	1

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: UNTREATED CONTROL

WEEKS ON STUDY	013	068	078	083	089	091	092	095	095	095	096	101	101	102	105	105	105	105	105	105	105	105	105	105	105
CARCASS ID	021	051	031	081	032	022	101	052	061	102	062	071	103	091	111	082	012	013	014	015	023	024	025	033	034
ALIMENTARY SYSTEM																									
Esophagus	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	M	+	A	A	M	+	+	+	+	+	+	+	+	+	M	+	M	+	+	M	+	+	+	+
Intestine large	+	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peyer's patch, lymphoma malignant lymphocytic																X									
Liver	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma			X		X		X			X	X												X		
Hepatocellular carcinoma, multiple																		X							
Hepatocellular adenoma																					X				
Lymphoma malignant lymphocytic																X									
Mesentery																+									
Lymphoma malignant lymphocytic																X									
Pancreas	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																									
Salivary glands	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																X									
Forestomach, papilloma squamous																									
Tooth												+													
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	A	+	M	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	I	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, carcinoma					X																				
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Epididymis	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland				+								+		+	+			+	M					+	
Prostate	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle																			+						
Testes	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Blood																			+						
Bone marrow	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin							X						X												
Sarcoma, metastatic, skin												X													
Bronchial, lymphoma malignant lymphocytic																								X	
Inguinal, lymphoma malignant lymphocytic																X									
Mandibular, lymphoma malignant lymphocytic																X									
Mediastinal, lymphoma malignant lymphocytic																X								X	
Mesenteric, lymphoma malignant lymphocytic																X								X	
Mesenteric, lymphoma malignant		X																							
Mesenteric, lymphoma malignant mixed																									
Pancreatic, lymphoma malignant mixed																									
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																				X					
Lymphoma malignant lymphocytic																X								X	
Lymphoma malignant mixed																									
Thymus	+	M	+	+	M	+	M	M	+	+	M	M	+	+	M	M	+	+	+	+	M	M	+	+	+
Lymphoma malignant lymphocytic																								X	

+: Tissue examined microscopically
: Not examined
−: Present but not examined microscopically
I: Insufficient tissue

M: Missing
A: Autolysis precludes examination
X: Incidence of listed morphology

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	035	041	042	043	044	045	053	054	055	063	064	065	072	073	074	075	083	084	085	092	093	094	095	104	105	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	M	+	M	M	M	M	M	M	M	M	+	+	+	M	+	+	+	+	M	+	+	+	31
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Peyer's patch, lymphoma malignant lymphocytic																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma					X															X		X				9
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma					X			X								X				X						5
Lymphoma malignant lymphocytic																										1
Mesentery			+																							2
Lymphoma malignant lymphocytic																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant lymphocytic																								X		1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant lymphocytic																										1
Forestomach, papilloma squamous	X																		X							2
Tooth																			+	+			+			4
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	M	+	+	M	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	44
Pituitary gland	+	+	M	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	M	+	+	41
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, carcinoma																										1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+										+					+				+		+			+	12
Prostate	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Seminal vesicle																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Blood																					+					2
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										2
Sarcoma, metastatic, skin																										1
Bronchial, lymphoma malignant lymphocytic																										1
Inguinal, lymphoma malignant lymphocytic																										1
Mandibular, lymphoma malignant lymphocytic																										1
Mediastinal, lymphoma malignant lymphocytic																										2
Mesenteric, lymphoma malignant lymphocytic																										2
Mesenteric, lymphoma malignant																										1
Mesenteric, lymphoma malignant mixed															X											1
Pancreatic, lymphoma malignant mixed															X											1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Lymphoma malignant lymphocytic																										2
Lymphoma malignant mixed															X											1
Thymus	+	+	M	+	M	M	+	+	+	+	+	+	M	M	M	+	M	+	+	M	M	+	+	+	+	31
Lymphoma malignant lymphocytic																										1

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	013	068	078	083	089	091	092	095	095	095	096	101	101	102	105	105	105	105	105	105	105	105	105	105	105
CARCASS ID	021	051	031	081	032	022	101	052	061	102	062	071	103	091	111	082	112	113	114	115	023	024	025	033	034
INTEGUMENTARY SYSTEM																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous													X												
Subcutaneous tissue, fibrosarcoma							X	X	X				X	X	X		X								
Subcutaneous tissue, fibrosarcoma, multiple																			X						
Subcutaneous tissue, hemangioma																									
Subcutaneous tissue, sarcoma				X						X		X													
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle															+										
Fibrosarcoma															X										
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma															X					X					
Alveolar/bronchiolar adenoma, multiple													X												
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, thyroid gland					X																				
Fibrosarcoma, metastatic, skin								X	X																
Hepatocellular carcinoma, metastatic, liver			X								X					X									
Lymphoma malignant lymphocytic																									
Nose	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Eye																			+	+	+				
Harderian gland																			+						+
Adenoma																			X						
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin								X																	
Cortex, renal tubule, adenoma																									
Urinary bladder	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
CARCASS ID	035	041	042	043	044	045	053	054	055	063	064	065	072	073	074	075	083	084	085	092	093	094	095	104	105	TOTAL: TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Mammary gland	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Papilloma squamous																										1
Subcutaneous tissue, fibrosarcoma		X													X											9
Subcutaneous tissue, fibrosarcoma, multiple																										1
Subcutaneous tissue, hemangioma					X																					1
Subcutaneous tissue, sarcoma												X														4
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Fibrosarcoma																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma									X				X								X					5
Alveolar/bronchiolar adenoma, multiple																										1
Alveolar/bronchiolar carcinoma											X			X				X								3
Carcinoma, metastatic, thyroid gland																										1
Fibrosarcoma, metastatic, skin																										2
Hepatocellular carcinoma, metastatic, liver																										2
Lymphoma malignant lymphocytic																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSES SYSTEM																										
Eye	+																					+				5
Harderian gland	+	+		+										+							+	+				8
Adenoma	X	X		X										X							X	X				7
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Cortex, renal tubule, adenoma													X													1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: LOW DOSE

WEEKS ON STUDY	062	069	069	072	078	082	089	090	091	092	093	095	095	096	100	101	101	104	105	105	105	105	105	105
CARCASS ID	271	301	231	251	221	232	261	291	222	211	241	302	212	252	242	243	292	272	223	253	283	213	214	215	224
ALIMENTARY SYSTEM																									
Esophagus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	M	+	+	+	+	A	M	M	M	M	M	M	M	M	A	+	+	+	+	+	+	+
Lymphoma malignant histiocytic								X																	
Intestine large	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine small	A	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+
Peyer's patch, lymphoma malignant mixed		X																							
Liver	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma														X	X	X				X					
Hepatocellular adenoma												X										X			X
Lymphoma malignant histiocytic											X	X													
Mesentery								+				+												+	
Lymphoma malignant histiocytic								X																	
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic								X																	
Salivary glands	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic								X																	
Forestomach, papilloma squamous																									
Tooth																									
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic								X																	
Cortex, adenoma																									
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	+	+	+	+	M	+	+	+	+	+	M	+	+	M	+	M	+	+	+	+	+	+	+	+
Pituitary gland	A	+	+	+	+	M	+	I	I	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
Follicular cell, carcinoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Epididymis	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland			M						+		+		+			+	+	+				M			+
Prostate	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle															+										
Testes	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Blood		M	M	M	M																				
Bone marrow	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin																					X				
Inguinal, lymphoma malignant histiocytic								X			X	X													
Mandibular, lymphoma malignant histiocytic								X				X													
Mediastinal, lymphoma malignant histiocytic												X													
Mesenteric, lymphoma malignant histiocytic											X	X													
Pancreatic, lymphoma malignant histiocytic												X													
Renal, lymphoma malignant histiocytic											X	X													
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic								X			X	X													
Thymus	+	+	M	M	+	+	+	+	M	M	+	+	M	+	+	+	M	+	M	+	+	+	+	+	+
Lymphoma malignant histiocytic								X				X													

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	225	233	234	235	244	245	254	255	262	263	264	265	273	274	275	281	282	284	285	293	294	295	303	304	305	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	M	+	+	+	+	+	M	+	+	+	+	M	+	+	M	+	+	M	M	M	+	+	+	M	30
Lymphoma malignant histiocytic																										1
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Peyer's patch, lymphoma malignant mixed																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma																		X						X		6
Hepatocellular adenoma				X		X					X													X		7
Lymphoma malignant histiocytic																										2
Mesentery			+												+											5
Lymphoma malignant histiocytic																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant histiocytic																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant histiocytic																										1
Forestomach, papilloma squamous																		X								1
Tooth									+					+								+				3
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant histiocytic																										1
Cortex, adenoma		X																								1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	M	M	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	M	+	40
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	44
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell, adenoma		X		X															X							3
Follicular cell, carcinoma							X																			1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+		+	+	+	+														+				+	+	15
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Seminal vesicle																			+							2
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																										
Blood																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Inguinal, lymphoma malignant histiocytic																										3
Mandibular, lymphoma malignant histiocytic																										2
Mediastinal, lymphoma malignant histiocytic																										1
Mesenteric, lymphoma malignant histiocytic																										2
Pancreatic, lymphoma malignant histiocytic																										1
Renal, lymphoma malignant histiocytic																										2
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant histiocytic																										3
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	40
Lymphoma malignant histiocytic																										2

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

WEEKS ON STUDY	062	069	069	072	078	082	089	090	091	092	093	095	095	096	100	101	101	104	105	105	105	105	105	105	105
CARCASS ID	271	301	231	251	221	232	261	291	222	211	241	302	212	252	242	243	292	272	223	253	283	213	214	215	224
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, fibroma																X									X
Subcutaneous tissue, fibrosarcoma						X				X			X				X		X		X				
Subcutaneous tissue, sarcoma									X		X														
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																				X					
Alveolar/bronchiolar carcinoma							X																		
Fibrosarcoma, metastatic, skin						X															X				
Hepatocellular carcinoma, metastatic, liver														X	X										
Lymphoma malignant histiocytic								X			X														
Nose	M	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Harderian gland																									
Adenoma																									
URINARY SYSTEM																									
Kidney	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic								X																	
Urethra									+																
Urinary bladder	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	225	233	234	235	244	245	254	255	262	263	264	265	273	274	275	281	282	284	285	293	294	295	303	304	305	TISSUES TUMORS
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, fibroma							X										X									4
Subcutaneous tissue, fibrosarcoma				X											X		X			X				X	X	12
Subcutaneous tissue, sarcoma																										2
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X				X					X									X					5
Alveolar/bronchiolar carcinoma											X															2
Fibrosarcoma, metastatic, skin																										2
Hepatocellular carcinoma, metastatic, liver																										2
Lymphoma malignant histiocytic																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Harderian gland				+															+							2
Adenoma				X															X							2
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant histiocytic																										1
Urethra																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: HIGH DOSE

WEEKS ON STUDY	054	055	056	057	062	063	075	100	100	100	101	101	105	105	105	105	105	105	105	105	105	105	105	105	105
CARCASS ID	191	111	192	181	161	193	201	202	112	115	121	131	113	114	122	123	124	125	132	133	134	135	141	142	143
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	A	A	M	A	+	+	M	M	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed																			X						
Intestine large	A	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ileum, Peyer's patch, lymphoma **malignant mixed**																			X						
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma							X			X															X
Hepatocellular carcinoma, multiple									X																
Hepatocellular adenoma																X									
Lymphoma malignant histiocytic											X														
Lymphoma malignant mixed																			X						
Mesentery	+								+		+														
Pancreas	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed																			X						
Salivary glands	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Forestomach, papilloma squamous																									
Tooth																									
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	M	+	M	M	M	+	+	M	+	+	+	+	+	+	+	M	+	+	M	+	+	+
Pituitary gland	I	+	I	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland									+	+	+		+		+	+	+		+	+		+		+	
Adenoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed																			X						
Seminal vesicle	+					+																			
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed																			X						
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inguinal, lymphoma malignant mixed																			X						
Mandibular, lymphoma malignant mixed																			X						
Mediastinal, lymphoma malignant mixed																			X						
Mesenteric, lymphoma malignant **histiocytic**											X														
Mesenteric, lymphoma malignant mixed																			X						
Pancreatic, lymphoma malignant mixed																			X						
Spleen	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic											X														
Lymphoma malignant lymphocytic																									X
Lymphoma malignant mixed																			X						
Thymus	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	144	145	151	152	153	154	155	162	163	164	165	171	172	173	174	175	182	183	184	185	194	195	203	204	205	TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	38
Lymphoma malignant mixed																										1
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Ileum, Peyer's patch, lymphoma malignant mixed																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma								X																X		5
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma				X	X		X																		X	5
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed																										1
Mesentery																	+							+		5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant mixed																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Forestomach, papilloma squamous															X											1
Tooth			+							+				+						+			+			5
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																									X	1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	41
Pituitary gland	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma														X							X		X			3
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+			+			+	+	+				+		+		+			+	+	25
Adenoma																			X							1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed																										1
Seminal vesicle																										2
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Inguinal, lymphoma malignant mixed																										1
Mandibular, lymphoma malig. mixed																										1
Mediastinal, lymphoma malig. mixed																										1
Mesenteric, lymphoma malignant histiocytic																										1
Mesenteric, lymphoma malignant mixed																										1
Pancreatic, lymphoma malignant mixed																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

WEEKS ON STUDY	054	055	056	057	062	063	075	100	100	100	101	101	105	105	105	105	105	105	105	105	105	105	105	105	105
CARCASS ID	191	111	192	181	161	193	201	202	112	115	121	131	113	114	122	123	124	125	132	133	134	135	141	142	143
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, fibroma																									
Subcutaneous tissue, fibrosarcoma					X													X				X			
Subcutaneous tissue, fibrosarcoma, multiple												X													
Subcutaneous tissue, sarcoma										X															
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
NERVOUS SYSTEM																									
Brain	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma													X												
Alveolar/bronchiolar carcinoma															X										
Fibrosarcoma, metastatic, skin					X																				
Lymphoma malignant histiocytic											X														
Nose	M	M	M	M	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Eye																									
Harderian gland																				+					
Adenoma																									
Lymphoma malignant mixed																				X					
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed																				X					
Urethra																									
Bulbourethral gland, leiomyosarcoma																									
Urinary bladder	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL:
CARCASS ID	144	145	151	152	153	154	155	162	163	164	165	171	172	173	174	175	182	183	184	185	194	195	203	204	205	TISSUES TUMORS
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, fibroma														X		X										2
Subcutaneous tissue, fibrosarcoma									X									X								5
Subcutaneous tissue, fibrosarcoma, multiple																										1
Subcutaneous tissue, sarcoma																										1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle				+																						2
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X																	X		X	4
Alveolar/bronchiolar carcinoma																										1
Fibrosarcoma, metastatic, skin																										1
Lymphoma malignant histiocytic																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Eye													+													1
Harderian gland									+				+													3
Adenoma									X				X													2
Lymphoma malignant mixed																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed																										1
Urethra					+																					1
Bulbourethral gland, leiomyosarcoma					X																					1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Control	1,000 ppm	2,000 ppm
Harderian Gland: Adenoma			
Overall Rates (a)	7/50 (14%)	2/50 (4%)	2/50 (4%)
Adjusted Rates (b)	19.4%	6.3%	5.3%
Terminal Rates (c)	7/36 (19%)	2/32 (6%)	2/38 (5%)
Day of First Observation	729	729	729
Life Table Tests (d)	P=0.037N	P=0.108N	P=0.067N
Logistic Regression Tests (d)	P=0.037N	P=0.108N	P=0.067N
Cochran-Armitage Trend Test (d)	P=0.042N		
Fisher Exact Test (d)		P=0.080N	P=0.080N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	5/49 (10%)	7/49 (14%)	5/50 (10%)
Adjusted Rates (b)	13.9%	20.8%	13.2%
Terminal Rates (c)	5/36 (14%)	6/32 (19%)	5/38 (13%)
Day of First Observation	729	663	729
Life Table Tests (d)	P=0.525N	P=0.299	P=0.597N
Logistic Regression Tests (d)	P=0.538N	P=0.323	P=0.597N
Cochran-Armitage Trend Test (d)	P=0.548N		
Fisher Exact Test (d)		P=0.380	P=0.617N
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	10/49 (20%)	6/49 (12%)	6/50 (12%)
Adjusted Rates (b)	23.2%	16.7%	14.3%
Terminal Rates (c)	5/36 (14%)	3/32 (9%)	3/38 (8%)
Day of First Observation	546	666	522
Life Table Tests (d)	P=0.162N	P=0.285N	P=0.207N
Logistic Regression Tests (d)	P=0.149N	P=0.205N	P=0.187N
Cochran-Armitage Trend Test (d)	P=0.151N		
Fisher Exact Test (d)		P=0.207N	P=0.194N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	13/49 (27%)	12/49 (24%)	11/50 (22%)
Adjusted Rates (b)	30.7%	32.8%	26.5%
Terminal Rates (c)	8/36 (22%)	8/32 (25%)	8/38 (21%)
Day of First Observation	546	663	522
Life Table Tests (d)	P=0.332N	P=0.559	P=0.379N
Logistic Regression Tests (d)	P=0.362N	P=0.509N	P=0.395N
Cochran-Armitage Trend Test (d)	P=0.342N		
Fisher Exact Test (d)		P=0.500N	P=0.385N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	6/50 (12%)	5/50 (10%)	4/50 (8%)
Adjusted Rates (b)	16.1%	15.6%	10.5%
Terminal Rates (c)	5/36 (14%)	5/32 (16%)	4/38 (11%)
Day of First Observation	705	729	729
Life Table Tests (d)	P=0.280N	P=0.582N	P=0.338N
Logistic Regression Tests (d)	P=0.291N	P=0.567N	P=0.360N
Cochran-Armitage Trend Test (d)	P=0.309N		
Fisher Exact Test (d)		P=0.500N	P=0.370N
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	8.3%	5.3%	2.6%
Terminal Rates (c)	3/36 (8%)	1/32 (3%)	1/38 (3%)
Day of First Observation	729	621	729
Life Table Tests (d)	P=0.216N	P=0.545N	P=0.286N
Logistic Regression Tests (d)	P=0.228N	P=0.514N	P=0.286N
Cochran-Armitage Trend Test (d)	P=0.222N		
Fisher Exact Test (d)		P=0.500N	P=0.309N

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Control	1,000 ppm	2,000 ppm
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	9/50 (18%)	7/50 (14%)	5/50 (10%)
Adjusted Rates (b)	24.2%	20.6%	13.2%
Terminal Rates (c)	8/36 (22%)	6/32 (19%)	5/38 (13%)
Day of First Observation	705	621	729
Life Table Tests (d)	P=0.136N	P=0.488N	P=0.164N
Logistic Regression Tests (d)	P=0.148N	P=0.452N	P=0.177N
Cochran-Armitage Trend Test (d)	P=0.157N		
Fisher Exact Test (d)		P=0.393N	P=0.194N
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted Rates (b)	0.0%	12.0%	5.3%
Terminal Rates (c)	0/36 (0%)	3/32 (9%)	2/38 (5%)
Day of First Observation		707	729
Life Table Tests (d)	P=0.244	P=0.052	P=0.250
Logistic Regression Tests (d)	P=0.224	P=0.058	P=0.250
Cochran-Armitage Trend Test (d)	P=0.222		
Fisher Exact Test (d)		P=0.059	P=0.247
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	10/50 (20%)	12/50 (24%)	6/50 (12%)
Adjusted Rates (b)	23.9%	32.3%	14.7%
Terminal Rates (c)	5/36 (14%)	8/32 (25%)	4/38 (11%)
Day of First Observation	639	573	433
Life Table Tests (d)	P=0.182N	P=0.310	P=0.200N
Logistic Regression Tests (d)	P=0.190N	P=0.388	P=0.209N
Cochran-Armitage Trend Test (d)	P=0.185N		
Fisher Exact Test (d)		P=0.405	P=0.207N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	10/50 (20%)	15/50 (30%)	8/50 (16%)
Adjusted Rates (b)	23.9%	39.8%	19.7%
Terminal Rates (c)	5/36 (14%)	10/32 (31%)	6/38 (16%)
Day of First Observation	639	573	433
Life Table Tests (d)	P=0.337N	P=0.126	P=0.374N
Logistic Regression Tests (d)	P=0.372N	P=0.161	P=0.404N
Cochran-Armitage Trend Test (d)	P=0.359N		
Fisher Exact Test (d)		P=0.178	P=0.398N
Subcutaneous Tissue: Sarcoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	9.4%	4.8%	2.4%
Terminal Rates (c)	1/36 (3%)	0/32 (0%)	0/38 (0%)
Day of First Observation	579	635	699
Life Table Tests (d)	P=0.126N	P=0.382N	P=0.182N
Logistic Regression Tests (d)	P=0.114N	P=0.339N	P=0.176N
Cochran-Armitage Trend Test (d)	P=0.118N		
Fisher Exact Test (d)		P=0.339N	P=0.181N
Subcutaneous Tissue: Sarcoma or Fibrosarcoma			
Overall Rates (a)	14/50 (28%)	14/50 (28%)	7/50 (14%)
Adjusted Rates (b)	31.6%	35.6%	16.7%
Terminal Rates (c)	6/36 (17%)	8/32 (25%)	4/38 (11%)
Day of First Observation	579	573	433
Life Table Tests (d)	P=0.073N	P=0.463	P=0.078N
Logistic Regression Tests (d)	P=0.063N	P=0.585	P=0.070N
Cochran-Armitage Trend Test (d)	P=0.062N		
Fisher Exact Test (d)		P=0.588N	P=0.070N

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Control	1,000 ppm	2,000 ppm
Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates (a)	14/50 (28%)	17/50 (34%)	9/50 (18%)
Adjusted Rates (b)	31.6%	42.7%	21.6%
Terminal Rates (c)	6/36 (17%)	10/32 (31%)	6/38 (16%)
Day of First Observation	579	573	433
Life Table Tests (d)	P=0.161N	P=0.244	P=0.172N
Logistic Regression Tests (d)	P=0.159N	P=0.326	P=0.173N
Cochran-Armitage Trend Test (d)	P=0.154N		
Fisher Exact Test (d)		P=0.333	P=0.171N
Thyroid Gland: Follicular Cell Adenoma			
Overall Rates (a)	0/50 (0%)	3/49 (6%)	3/50 (6%)
Adjusted Rates (b)	0.0%	9.4%	7.9%
Terminal Rates (c)	0/36 (0%)	3/32 (9%)	3/38 (8%)
Day of First Observation		729	729
Life Table Tests (d)	P=0.116	P=0.101	P=0.131
Logistic Regression Tests (d)	P=0.116	P=0.101	P=0.131
Cochran-Armitage Trend Test (d)	P=0.102		
Fisher Exact Test (d)		P=0.117	P=0.121
Thyroid Gland: Follicular Cell Adenoma or Carcinoma			
Overall Rates (a)	0/50 (0%)	4/49 (8%)	3/50 (6%)
Adjusted Rates (b)	0.0%	12.5%	7.9%
Terminal Rates (c)	0/36 (0%)	4/32 (13%)	3/38 (8%)
Day of First Observation		729	729
Life Table Tests (d)	P=0.135	P=0.049	P=0.131
Logistic Regression Tests (d)	P=0.135	P=0.049	P=0.131
Cochran-Armitage Trend Test (d)	P=0.119		
Fisher Exact Test (d)		P=0.056	P=0.121
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	5/50 (10%)	4/50 (8%)	3/50 (6%)
Adjusted Rates (b)	12.9%	9.1%	7.6%
Terminal Rates (c)	4/36 (11%)	0/32 (0%)	2/38 (5%)
Day of First Observation	474	479	705
Life Table Tests (d)	P=0.293N	P=0.545N	P=0.342N
Logistic Regression Tests (d)	P=0.280N	P=0.508N	P=0.359N
Cochran-Armitage Trend Test (d)	P=0.290N		
Fisher Exact Test (d)		P=0.500N	P=0.357N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors 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. A negative trend or lower incidence in a dosed group is indicated by (N).

TABLE C4. HISTORICAL INCIDENCE OF THYROID GLAND FOLLICULAR CELL TUMORS IN MALE $B6C3F_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls	
	Adenoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute		
HC Blue No. 2	2/44	2/44
C.I. Disperse Blue 1	2/49	2/49
D-Mannitol	0/50	0/50
Ziram	2/49	2/49
Eugenol	0/48	0/48
Propyl gallate	3/49	3/49
Zearalenone	2/50	2/50
HC Blue No. 1	0/47	0/47
Stannous chloride	0/48	0/48
TOTAL	11/434 (2.5%)	11/434 (2.5%)
SD (b)	2.49%	2.49%
Range (c)		
High	3/49	3/49
Low	0/50	0/50
Overall Historical Incidence		
TOTAL	(d) 26/1,958 (1.3%)	(d) 29/1,958 (1.5%)
SD (b)	1.98%	2.01%
Range (c)		
High	3/42	3/42
Low	0/50	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) Includes one papillary adenoma and one cystadenoma, NOS

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Intestine small	(46)	(46)	(45)
Ileum, Peyer's patch, hyperplasia, lymphoid	2 (4%)		1 (2%)
Liver	(49)	(49)	(50)
Angiectasis		1 (2%)	1 (2%)
Basophilic focus	2 (4%)		
Cyst	3 (6%)	1 (2%)	
Cytoplasmic alteration, focal	1 (2%)		
Hematopoietic cell proliferation	1 (2%)	3 (6%)	2 (4%)
Hemorrhage, chronic			1 (2%)
Infiltration cellular, lymphocytic	2 (4%)		
Inflammation, granulomatous			1 (2%)
Mineralization		1 (2%)	
Necrosis	2 (4%)	4 (8%)	1 (2%)
Pigmentation, hemosiderin			2 (4%)
Mesentery	(2)	(5)	(5)
Hemorrhage	1 (50%)		
Fat, necrosis, focal		3 (60%)	4 (80%)
Pancreas	(48)	(49)	(48)
Acinus, atrophy, multifocal		1 (2%)	
Acinus, hyperplasia		1 (2%)	
Salivary glands	(49)	(49)	(49)
Cyst	1 (2%)		
Stomach	(48)	(49)	(50)
Forestomach, cyst		1 (2%)	1 (2%)
Forestomach, edema	1 (2%)		
Forestomach, inflammation, suppurative		1 (2%)	
Forestomach, ulcer			1 (2%)
Glandular, mineralization			1 (2%)
Tooth	(4)	(3)	(5)
Dysplasia	4 (100%)	3 (100%)	2 (40%)
Inflammation, chronic		1 (33%)	
Inflammation, suppurative	1 (25%)	2 (67%)	3 (60%)
CARDIOVASCULAR SYSTEM			
Heart	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)		
ENDOCRINE SYSTEM			
Adrenal gland	(49)	(48)	(49)
Cortex, hyperplasia, focal		1 (2%)	
Medulla, hyperplasia, focal	1 (2%)		
Medulla, hyperplasia, multifocal			1 (2%)
Spindle cell, hyperplasia, focal	4 (8%)	1 (2%)	
Spindle cell, hyperplasia, multifocal	1 (2%)		
Parathyroid gland	(44)	(40)	(41)
Cyst			1 (2%)
Thyroid gland	(50)	(49)	(50)
Degeneration, cystic	13 (26%)	12 (24%)	9 (18%)
Hyperplasia, cystic	3 (6%)	2 (4%)	5 (10%)
Infiltration cellular, lymphocytic	2 (4%)		
C-cell, hyperplasia, focal			1 (2%)
Follicle, cyst	2 (4%)	1 (2%)	1 (2%)
Follicular cell, hyperplasia, focal	3 (6%)	4 (8%)	1 (2%)

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Epididymis	(49)	(49)	(50)
Inflammation, suppurative, diffuse		1 (2%)	
Preputial gland	(12)	(15)	(25)
Fibrosis			1 (4%)
Infiltration cellular, lymphocytic	1 (8%)		2 (8%)
Inflammation, chronic	1 (8%)		2 (8%)
Inflammation, granulomatous			3 (12%)
Inflammation, suppurative	6 (50%)	10 (67%)	8 (32%)
Metaplasia, osseous			1 (4%)
Mineralization	1 (8%)		2 (8%)
Duct, ectasia	7 (58%)	12 (80%)	17 (68%)
Duct, inflammation, suppurative	1 (8%)		
Prostate	(47)	(48)	(50)
Inflammation, granulomatous		1 (2%)	
Inflammation, suppurative		1 (2%)	
Seminal vesicle	(1)	(2)	(2)
Atrophy	1 (100%)		
Inflammation, suppurative		1 (50%)	
Testes	(49)	(47)	(50)
Atrophy	1 (2%)		
Mineralization		1 (2%)	3 (6%)
HEMATOPOIETIC SYSTEM			
Bone marrow	(49)	(49)	(50)
Angiectasis	1 (2%)		
Atrophy			1 (2%)
Myelofibrosis	1 (2%)		
Myeloid cell, hyperplasia	9 (18%)	7 (14%)	5 (10%)
Lymph node	(50)	(50)	(50)
Inguinal, hyperplasia, lymphoid	2 (4%)	2 (4%)	9 (18%)
Inguinal, hyperplasia, plasma cell	1 (2%)		
Inguinal, necrosis		1 (2%)	
Inguinal, pigmentation	2 (4%)		1 (2%)
Inguinal, renal, iliac, autolysis	1 (2%)		
Mandibular, hyperplasia, lymphoid	2 (4%)		2 (4%)
Mandibular, pigmentation			1 (2%)
Mesenteric, angiectasis	13 (26%)	8 (16%)	3 (6%)
Mesenteric, hematopoietic cell proliferation	7 (14%)	1 (2%)	1 (2%)
Mesenteric, hyperplasia, lymphoid	8 (16%)	2 (4%)	2 (4%)
Mesenteric, syncytial alteration	1 (2%)		
Pancreatic, hyperplasia, lymphoid			1 (2%)
Renal, hyperplasia	1 (2%)		
Spleen	(49)	(49)	(48)
Atrophy	1 (2%)	1 (2%)	
Hematopoietic cell proliferation	14 (29%)	13 (27%)	6 (13%)
Hyperplasia, lymphoid	3 (6%)	1 (2%)	2 (4%)
Lymphoid follicle, amyloid deposition	1 (2%)		
Thymus	(31)	(40)	(47)
Hyperplasia, lymphoid		1 (3%)	
Necrosis		1 (3%)	

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
INTEGUMENTARY SYSTEM			
Skin	(48)	(50)	(50)
Cyst epithelial inclusion		1 (2%)	
Fibrosis, focal	17 (35%)	25 (50%)	24 (48%)
Hemorrhage, multifocal		1 (2%)	
Hyperplasia, focal	1 (2%)	1 (2%)	
Inflammation, chronic		1 (2%)	
Inflammation, chronic, focal	2 (4%)		4 (8%)
Inflammation, suppurative, focal	3 (6%)	2 (4%)	1 (2%)
Mineralization	1 (2%)	1 (2%)	1 (2%)
Necrosis, focal	1 (2%)	1 (2%)	
Ulcer, focal	5 (10%)	4 (8%)	1 (2%)
Subcutaneous tissue, abscess, chronic	1 (2%)		1 (2%)
Subcutaneous tissue, edema			1 (2%)
MUSCULOSKELETAL SYSTEM			
Skeletal muscle	(1)		(2)
Inflammation, suppurative			1 (50%)
NERVOUS SYSTEM			
Brain	(50)	(49)	(49)
Corpora amylacea	2 (4%)	12 (24%)	10 (20%)
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(50)
Congestion		1 (2%)	1 (2%)
Embolus tumor		1 (2%)	
Hemorrhage	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocytic	1 (2%)		1 (2%)
Necrosis, diffuse			1 (2%)
Necrosis, multifocal		1 (2%)	
Alveolar epithelium, hyperplasia, diffuse	1 (2%)	1 (2%)	
Alveolar epithelium, hyperplasia, focal	3 (6%)	1 (2%)	
Alveolar epithelium, hyperplasia, multifocal	1 (2%)	1 (2%)	1 (2%)
Bronchus, foreign body			1 (2%)
Bronchus, inflammation, suppurative			1 (2%)
Nose	(48)	(46)	(43)
Lumen, foreign body		1 (2%)	5 (12%)
Lumen, inflammation, suppurative	1 (2%)		5 (12%)
Nasolacrimal duct, inflammation, suppurative	1 (2%)		
Submucosa, sinus, inflammation, granulomatous, suppurative		1 (2%)	
SPECIAL SENSES SYSTEM			
None			

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM			
Kidney	(50)	(49)	(50)
Bacterium			2 (4%)
Degeneration, focal		1 (2%)	
Hydronephrosis		1 (2%)	
Infarct		1 (2%)	1 (2%)
Infiltration cellular, lymphocytic	23 (46%)	23 (47%)	18 (36%)
Inflammation, suppurative		2 (4%)	2 (4%)
Nephropathy, chronic	38 (76%)	34 (69%)	42 (84%)
Cortex, cyst		1 (2%)	
Cortex, fibrosis			1 (2%)
Corticomedullary junction, fibrosis		1 (2%)	
Interstitial tissue, mineralization	37 (74%)	34 (69%)	40 (80%)
Interstitial tissue, pigmentation			1 (2%)
Medulla, cyst		1 (2%)	
Renal tubule, dilatation		1 (2%)	
Urethra		(1)	(1)
Inflammation		1 (100%)	
Urinary bladder	(48)	(48)	(48)
Inflammation, proliferative			1 (2%)
Mucosa, inflammation, suppurative		1 (2%)	

APPENDIX D

SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Esophagus	(50)	(50)	(48)
Lymphoma malignant lymphocytic			1 (2%)
Intestine small	(49)	(45)	(48)
Lymphoma malignant mixed	2 (4%)		
Ileum, Peyer's patch, lymphoma malignant lymphocytic		1 (2%)	3 (6%)
Ileum, Peyer's patch, lymphoma malignant mixed	1 (2%)	1 (2%)	
Jejunum, polyp adenomatous	1 (2%)		
Jejunum, Peyer's patch, lymphoma malignant mixed	1 (2%)		1 (2%)
Wall, lymphoma malignant mixed	1 (2%)		
Liver	(50)	(50)	(49)
Hemangiosarcoma	1 (2%)		
Hemangiosarcoma, metastatic, spleen			1 (2%)
Hepatocellular carcinoma	3 (6%)	1 (2%)	4 (8%)
Hepatocellular adenoma	5 (10%)	3 (6%)	1 (2%)
Lymphoma malignant histiocytic	1 (2%)	2 (4%)	
Lymphoma malignant lymphocytic	3 (6%)		2 (4%)
Lymphoma malignant mixed	1 (2%)	1 (2%)	3 (6%)
Mesentery	*(50)	*(50)	*(50)
Lymphoma malignant histiocytic	1 (2%)		
Lymphoma malignant lymphocytic			2 (4%)
Lymphoma malignant mixed	3 (6%)		
Sarcoma stromal, metastatic, focal			1 (2%)
Pancreas	(49)	(48)	(47)
Lymphoma malignant mixed	2 (4%)		
Salivary glands	(49)	(48)	(48)
Lymphoma malignant lymphocytic			1 (2%)
Lymphoma malignant mixed		1 (2%)	1 (2%)
Stomach	(49)	(48)	(47)
Lymphoma malignant lymphocytic			1 (2%)
Forestomach, papilloma squamous	1 (2%)	1 (2%)	
CARDIOVASCULAR SYSTEM			
Heart	(50)	(50)	(49)
Lymphoma malignant lymphocytic	1 (2%)		
ENDOCRINE SYSTEM			
Adrenal gland	(49)	(49)	(49)
Lymphoma malignant lymphocytic			1 (2%)
Pheochromocytoma benign			1 (2%)
Cortex, lymphoma malignant mixed			1 (2%)
Islets, pancreatic	(50)	(48)	(48)
Adenoma		1 (2%)	2 (4%)
Carcinoma		1 (2%)	
Pituitary gland	(49)	(44)	(46)
Pars distalis, adenoma	4 (8%)	4 (9%)	6 (13%)
Pars distalis, carcinoma		1 (2%)	
Thyroid gland	(50)	(50)	(48)
Lymphoma malignant lymphocytic			1 (2%)
Bilateral, follicular cell, adenoma		1 (2%)	
Follicular cell, adenoma	1 (2%)	1 (2%)	
Follicular cell, adenoma, multiple			1 (2%)

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Ovary	(48)	(45)	(48)
Lymphoma malignant histiocytic		1 (2%)	
Lymphoma malignant mixed		1 (2%)	
Bilateral, cystadenoma		1 (2%)	
Uterus	(50)	(50)	(49)
Hemangiosarcoma, metastatic, spleen			1 (2%)
Leiomyoma		2 (4%)	
Lymphoma malignant histiocytic	1 (2%)		
Lymphoma malignant lymphocytic			1 (2%)
Lymphoma malignant mixed	2 (4%)		
Polyp stromal	1 (2%)		
Sarcoma	1 (2%)		1 (2%)
Sarcoma stromal	1 (2%)	1 (2%)	1 (2%)
Vagina	*(50)	*(50)	*(50)
Squamous cell carcinoma		1 (2%)	
HEMATOPOIETIC SYSTEM			
Bone marrow	(48)	(48)	(49)
Hemangioma	1 (2%)		
Hemangiosarcoma, metastatic, spleen			1 (2%)
Lymphoma malignant mixed		1 (2%)	1 (2%)
Lymph node	(50)	(50)	(49)
Carcinoma, metastatic, harderian gland		1 (2%)	
Fibrosarcoma, metastatic, skin		1 (2%)	
Sarcoma, metastatic, skeletal muscle			1 (2%)
Axillary, lymphoma malignant mixed			1 (2%)
Bronchial, lymphoma malignant lymphocytic			1 (2%)
Bronchial, lymphoma malignant mixed			2 (4%)
Iliac, lymphoma malignant lymphocytic	3 (6%)	1 (2%)	
Iliac, lymphoma malignant mixed	1 (2%)		
Inguinal, lymphoma malignant mixed			1 (2%)
Lumbar, lymphoma malignant lymphocytic	1 (2%)		
Mandibular, lymphoma malignant histiocytic		2 (4%)	
Mandibular, lymphoma malignant lymphocytic	4 (8%)	1 (2%)	2 (4%)
Mandibular, lymphoma malignant mixed	2 (4%)	1 (2%)	2 (4%)
Mediastinal, lymphoma malignant lymphocytic	3 (6%)		2 (4%)
Mediastinal, lymphoma malignant mixed	4 (8%)	1 (2%)	2 (4%)
Mediastinal, mesenteric, lymphoma malignant lymphocytic	1 (2%)		
Mesenteric, lymphoma malignant histiocytic		1 (2%)	
Mesenteric, lymphoma malignant lymphocytic	2 (4%)	1 (2%)	3 (6%)
Mesenteric, lymphoma malignant mixed	3 (6%)	2 (4%)	3 (6%)
Pancreatic, lymphoma malignant histiocytic		1 (2%)	
Pancreatic, lymphoma malignant mixed	1 (2%)		2 (4%)
Renal, lymphoma malignant histiocytic	1 (2%)		
Renal, lymphoma malignant lymphocytic	3 (6%)	1 (2%)	
Renal, lymphoma malignant mixed	1 (2%)	1 (2%)	
Spleen	(49)	(49)	(49)
Hemangiosarcoma	1 (2%)		1 (2%)
Lymphoma malignant histiocytic	1 (2%)	2 (4%)	
Lymphoma malignant lymphocytic	6 (12%)	1 (2%)	2 (4%)
Lymphoma malignant mixed	5 (10%)	4 (8%)	3 (6%)
Thymus	(48)	(48)	(40)
Lymphoma malignant histiocytic		1 (2%)	
Lymphoma malignant lymphocytic	1 (2%)	1 (2%)	1 (3%)
Lymphoma malignant mixed		1 (2%)	

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
INTEGUMENTARY SYSTEM			
Mammary gland	(46)	(47)	(49)
Adenocarcinoma		2 (4%)	2 (4%)
Lymphoma malignant lymphocytic			1 (2%)
Skin	(50)	(50)	(49)
Hair follicle, lymphoma malignant lymphocytic	1 (2%)		
Subcutaneous tissue, fibrosarcoma		1 (2%)	1 (2%)
Subcutaneous tissue, lymphoma malignant lymphocytic			1 (2%)
Tail, papilloma squamous			1 (2%)
MUSCULOSKELETAL SYSTEM			
Skeletal muscle	*(50)	*(50)	*(50)
Fibrosarcoma	1 (2%)		
Sarcoma			1 (2%)
NERVOUS SYSTEM			
Brain	(50)	(48)	(49)
Lymphoma malignant lymphocytic	1 (2%)		
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(49)
Alveolar/bronchiolar adenoma	3 (6%)	5 (10%)	3 (6%)
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)	
Carcinoma, metastatic, harderian gland		1 (2%)	
Hepatocellular carcinoma, metastatic, liver	1 (2%)		
Lymphoma malignant histiocytic	1 (2%)	1 (2%)	
Lymphoma malignant lymphocytic	2 (4%)		1 (2%)
Lymphoma malignant mixed		1 (2%)	1 (2%)
Sarcoma		1 (2%)	
Sarcoma, metastatic, skeletal muscle			1 (2%)
Capillary, lymphoma malignant histiocytic		1 (2%)	
Capillary, lymphoma malignant lymphocytic	1 (2%)		
Mediastinum, lymphoma malignant lymphocytic			1 (2%)
SPECIAL SENSES SYSTEM			
Harderian gland	*(50)	*(50)	*(50)
Adenoma		4 (8%)	
Carcinoma		1 (2%)	
URINARY SYSTEM			
Kidney	(50)	(50)	(49)
Lymphoma malignant histiocytic		1 (2%)	
Lymphoma malignant lymphocytic	4 (8%)		2 (4%)
Lymphoma malignant mixed	1 (2%)	1 (2%)	1 (2%)
Urinary bladder	(48)	(47)	(49)
Lymphoma malignant lymphocytic	1 (2%)		1 (2%)
Lymphoma malignant mixed	1 (2%)		
SYSTEMIC LESIONS			
Multiple organs	*(50)	*(50)	*(50)
Lymphoma malignant mixed	9 (18%)	4 (8%)	3 (6%)
Hemangiosarcoma	2 (4%)		1 (2%)
Lymphoma malignant histiocytic	1 (2%)	2 (4%)	
Lymphoma malignant lymphocytic	6 (12%)	2 (4%)	4 (8%)
Hemangioma	1 (2%)		

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Dead	8	7	6
Terminal sacrifice	39	35	35
Moribund	3	8	7
Terminal sacrifice			1
Natural death			1
TUMOR SUMMARY			
Total animals with primary neoplasms **	29	33	25
Total primary neoplasms	42	42	33
Total animals with benign neoplasms	13	20	11
Total benign neoplasms	17	23	15
Total animals with malignant neoplasms	21	17	18
Total malignant neoplasms	25	19	18
Total animals with secondary neoplasms ***	1	2	3
Total secondary neoplasms	1	3	6

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
*** Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: UNTREATED CONTROL

WEEKS ON STUDY	0 8 2	0 8 5	0 9 0	0 9 4	0 9 5	0 9 5	0 9 8	0 9 9	1 0 0	1 0 3	1 0 4	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5
CARCASS ID	3 3 1	3 5 1	3 1 1	3 3 2	3 8 1	3 1 2	3 4 1	3 7 1	3 2 1	3 7 2	4 0 1	3 1 3	3 1 4	3 1 5	3 2 2	3 2 3	3 6 1	4 0 2	3 2 4	3 2 5	3 3 3	3 3 4	3 3 5	3 4 2	3 4 3
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	A	+	M	M	+	+	M	M	M	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed											X						X								
Ileum, Peyer's patch, lymphoma malignant mixed																									
Jejunum, polyp adenomatous																									
Jejunum, Peyer's patch, lymphoma malignant mixed									X																
Wall, lymphoma malignant mixed			X																						
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Hepatocellular carcinoma									X																
Hepatocellular adenoma													X											X	
Lymphoma malignant histiocytic				X																					
Lymphoma malignant lymphocytic							X																		
Lymphoma malignant mixed																									
Mesentery		+	+	+		+		+		+							+	+							
Lymphoma malignant histiocytic				X																					
Lymphoma malignant mixed			X			X											X								
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed			X			X																			
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Forestomach, papilloma squamous																									
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic							X																		
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma													X					X							
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic				X																					
Lymphoma malignant mixed			X			X																			
Polyp stromal																									
Sarcoma																									
Sarcoma stromal				X																					

+ Tissue examined microscopically
Not examined
– Present but not examined microscopically
I Insufficient tissue

M Missing
A Autolysis precludes examination
X Incidence of listed morphology

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
CARCASS ID	344	345	352	353	354	355	362	363	364	365	373	374	375	382	383	384	385	391	392	393	394	395	403	404	405	TOTAL TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	M	+	+	+	+	+	M	+	M	+	+	+	M	+	+	+	+	+	+	37
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant mixed																										2
Ileum, Peyer's patch, lymphoma malignant mixed					X																					1
Jejunum, polyp adenomatous																								X		1
Jejunum, Peyer's patch, lymphoma malignant mixed																										1
Wall, lymphoma malignant mixed																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																				X						1
Hepatocellular carcinoma																			X	X						3
Hepatocellular adenoma											X	X													X	5
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic															X		X									3
Lymphoma malignant mixed					X																					1
Mesentery					+								+			+										11
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed																										3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant mixed																										2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Forestomach, papilloma squamous							X																			1
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma																								X	X	4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																				X						1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed																										2
Polyp stromal															X											1
Sarcoma				X																						1
Sarcoma stromal																										1

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	082	085	090	094	095	095	098	099	100	103	104	105	105	105	105	105	105	105	105	105	105	105	105	105	105
CARCASS ID	331	351	311	332	381	312	341	371	321	372	401	313	314	315	322	323	361	402	324	325	333	334	335	342	343
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Iliac, lymphoma malignant lymphocytic																									
Iliac, lymphoma malignant mixed			X																						
Lumbar, lymphoma malignant lymphocytic																									
Mandibular, lymphoma malignant lymphocytic																									
Mandibular, lymphoma malignant mixed			X																						
Mediastinal, lymphoma malignant lymphocytic																									
Mediastinal, lymphoma malignant mixed			X			X			X																
Mediastinal, mesenteric, lymphoma malignant lymphocytic							X																		
Mesenteric, lymphoma malignant lymphocytic																									
Mesenteric, lymphoma malignant mixed			X						X								X								
Pancreatic, lymphoma malignant mixed																	X								
Renal, lymphoma malignant histiocytic				X																					
Renal, lymphoma malignant lymphocytic																									
Renal, lymphoma malignant mixed								X																	
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																X									
Lymphoma malignant histiocytic				X																					
Lymphoma malignant lymphocytic							X																	X	
Lymphoma malignant mixed			X														X								
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																									
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hair follicle, lymphoma malignant lymphocytic							X																		
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle					+																				
Fibrosarcoma					X																				
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic							X																		
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																					X				X
Alveolar/bronchiolar carcinoma								X																	
Hepatocellular carcinoma, metastatic, liver																									
Lymphoma malignant histiocytic				X																					
Lymphoma malignant lymphocytic																								X	
Capillary, lymphoma malignant lymphocytic							X																		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Harderian gland																					+				
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic							X																		
Lymphoma malignant mixed						X																			
Urinary bladder	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																									
Lymphoma malignant mixed			X																						

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: UNTREATED CONTROL (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL TISSUES TUMORS
CARCASS ID	3444	3445	3452	3533	3544	3555	3562	3633	3644	3655	3733	3741	3755	3822	3833	3841	3855	3911	3922	3933	3944	3955	4003	4044	4055	
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	48
Hemangioma																					X					1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Iliac, lymphoma malig lymphocytic									X	X					X											3
Iliac, lymphoma malignant mixed																										1
Lumbar, lymphoma malig lymphocytic									X																	1
Mandibular, lymphoma malignant lymphocytic									X	X					X		X									4
Mandibular, lymphoma malig mixed					X																					2
Mediastinal, lymphoma malignant lymphocytic									X	X							X									3
Mediastinal, lymphoma malig mixed					X																					4
Mediastinal, mesenteric, lymphoma malignant lymphocytic																										1
Mesenteric, lymphoma malignant lymphocytic									X						X											2
Mesenteric, lymphoma malignant mixed																										3
Pancreatic, lymphoma malignant mixed																										1
Renal, lymphoma malignant histiocytic																										1
Renal, lymphoma malig lymphocytic									X	X					X											3
Renal, lymphoma malignant mixed																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic									X	X					X		X									6
Lymphoma malignant mixed					X		X												X							5
Thymus	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant lymphocytic									X																	1
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	46
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hair follicle, lymphoma malignant lymphocytic																										1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Fibrosarcoma																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																				X						3
Alveolar/bronchiolar carcinoma																										1
Hepatocellular carcinoma, metastatic, liver																					X					1
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic																	X									2
Capillary, lymphoma malignant lymphocytic																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Harderian gland																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic									X	X							X									4
Lymphoma malignant mixed																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant lymphocytic																	X									1
Lymphoma malignant mixed																										1

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: LOW DOSE

WEEKS ON STUDY	063	078	083	083	092	093	096	096	096	097	099	100	101	102	104	105	105	105	105	105	105	105	105	105	105
CARCASS ID	60 1	58 1	57 1	59 1	57 2	52 1	53 1	60 2	60 3	55 1	51 1	52 2	56 1	52 3	54 1	58 2	51 2	51 3	51 4	51 5	52 4	52 5	53 2	53 3	53 4
ALIMENTARY SYSTEM																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	+	A	A	A	A	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	M	+	+	M
Intestine large	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	A	A	+	A	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+
Ileum, Peyer's patch, lymphoma malignant lymphocytic													X												
Ileum, Peyer's patch, lymphoma malignant mixed															X										
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma				X																					
Hepatocellular adenoma											X												X		
Lymphoma malignant histiocytic	X															X									
Lymphoma malignant mixed															X										
Mesentery												+													
Pancreas	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed															X										
Stomach	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Forestomach, papilloma squamous																						X			
Tooth																									
CARDIOVASCULAR SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma		X																							
Carcinoma																									
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Pituitary gland	+	+	A	+	+	A	+	+	+	+	+	+	+	I	M	+	I	+	+	+	+	+	+	+	+
Pars distalis, adenoma											X										X				
Pars distalis, carcinoma								X																	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, follicular cell, adenoma																									
Follicular cell, adenoma																								X	
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Ovary	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	M	+	M
Lymphoma malignant histiocytic																X									
Lymphoma malignant mixed															X										
Bilateral, cystadenoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma													X												
Sarcoma stromal																									
Vagina																									
Squamous cell carcinoma																									

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
CARCASS ID	535	542	543	544	545	552	553	554	555	562	563	564	565	573	574	575	583	584	585	592	593	594	595	604	605	TOTAL TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Ileum, Peyer's patch, lymphoma malignant lymphocytic																										1
Ileum, Peyer's patch, lymphoma malignant mixed																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																										1
Hepatocellular adenoma					X																					3
Lymphoma malignant histiocytic																										2
Lymphoma malignant mixed																										1
Mesentery			+																+							3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant mixed																										1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Forestomach, papilloma squamous																										1
Tooth																							+			1
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																										1
Carcinoma																									X	1
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Pars distalis, adenoma											X							X								4
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, follicular cell, adenoma																		X								1
Follicular cell, adenoma																										1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Ovary	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	45
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed																										1
Bilateral, cystadenoma									X																	1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma			X																							2
Sarcoma stromal										X																1
Vagina													+													1
Squamous cell carcinoma													X													1

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

WEEKS ON STUDY	063	078	083	083	092	093	096	096	096	097	099	100	101	102	104	105	105	105	105	105	105	105	105	105	105
CARCASS ID	601	581	571	591	572	521	531	602	603	551	511	522	561	523	541	582	512	513	514	515	524	525	532	533	534
HEMATOPOIETIC SYSTEM																									
Blood		M																							
Bone marrow	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant mixed															X										
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, harderian gland																									
Fibrosarcoma, metastatic, skin					X																				
Iliac, lymphoma malignant lymphocytic																		X							
Mandibular, lymphoma malignant histiocytic	X															X									
Mandibular, lymphoma malignant lymphocytic													X												
Mandibular, lymphoma malignant mixed															X										
Mediastinal, lymphoma malignant mixed															X										
Mesenteric, lymphoma malignant histiocytic																X									
Mesenteric, lymphoma malignant lymphocytic													X												
Mesenteric, lymphoma malignant mixed															X										
Pancreatic, lymphoma malignant histiocytic																X									
Renal, lymphoma malignant lymphocytic																		X							
Renal, lymphoma malignant mixed																									
Spleen	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic	X															X									
Lymphoma malignant lymphocytic																		X							
Lymphoma malignant mixed															X										
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic	X																								
Lymphoma malignant lymphocytic																		X							
Lymphoma malignant mixed															X										
INTEGUMENTARY SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Adenocarcinoma											X														
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma					X																				
MUSCULOSKELETAL SYSTEM																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
RESPIRATORY SYSTEM																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X															X	
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, harderian gland																									
Lymphoma malignant histiocytic	X																								
Lymphoma malignant mixed															X										
Sarcoma			X																						
Capillary, lymphoma malignant histiocytic																X									
Nose	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
Harderian gland															+										
Adenoma															X										
Carcinoma																									
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic	X																								
Lymphoma malignant mixed															X										
Urinary bladder	+	+	+	+	I	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL
CARCASS ID	535	542	543	544	545	552	553	554	555	562	563	564	565	573	574	575	583	584	585	592	593	594	595	604	605	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Blood																							+			1
Bone marrow	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant mixed																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, harderian gland																								X		1
Fibrosarcoma, metastatic, skin																										1
Iliac, lymphoma malignant lymphocytic																										1
Mandibular, lymphoma malignant histiocytic																										2
Mandibular, lymphoma malignant lymphocytic																										1
Mandibular, lymphoma malig mixed																										1
Mediastinal, lymphoma malig mixed																										1
Mesenteric, lymphoma malignant histiocytic																										1
Mesenteric, lymphoma malignant lymphocytic																										1
Mesenteric, lymphoma malig mixed							X																			2
Pancreatic, lymphoma malignant histiocytic																										1
Renal, lymphoma malig lymphocytic																										1
Renal, lymphoma malignant mixed							X																			1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant histiocytic																										2
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed							X																X	X		4
Thymus	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																										1
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	47
Adenocarcinoma												X														2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																		+								1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																	X		X						X	5
Alveolar/bronchiolar carcinoma																								X		1
Carcinoma, metastatic, harderian gland																								X		1
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed																										1
Sarcoma																										1
Capillary, lymphoma malignant histiocytic																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSES SYSTEM																										
Harderian gland										+				+	+									+		5
Adenoma										X				X	X											4
Carcinoma																								X		1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: HIGH DOSE

WEEKS ON STUDY	06 6	08 4	08 6	08 8	09 1	09 2	09 2	09 8	10 0	10 0	10 0	10 0	10 4	10 4	10 5	10 5	10 5	10 5	10 5	10 5	10 5	10 5	10 5	10 5	10 5
CARCASS ID	431	411	412	481	491	432	492	482	441	421	471	451	435	493	434	413	414	415	422	423	424	425	433	442	443
ALIMENTARY SYSTEM																									
Esophagus	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic														X											
Gallbladder	+	A	+	A	+	M	M	M	M	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ileum, Peyer's patch, lymphoma malignant lymphocytic															X		X								
Jejunum, Peyer's patch, lymphoma malignant mixed						X																			
Liver	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, spleen													X												
Hepatocellular carcinoma																					X	X			
Hepatocellular adenoma																	X								
Lymphoma malignant lymphocytic																		X							
Lymphoma malignant mixed	X					X						X													
Mesentery			+	+			+	+	+	+				+											
Lymphoma malignant lymphocytic														X											
Sarcoma stromal, metastatic, focal										X															
Pancreas	+	A	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	A	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic														X											
Lymphoma malignant mixed						X																			
Stomach	+	A	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic														X											
CARDIOVASCULAR SYSTEM																									
Heart	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Adrenal gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic														X											
Pheochromocytoma benign																				X					
Cortex, lymphoma malignant mixed						X																			
Islets, pancreatic	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X				X								
Parathyroid gland	M	A	+	M	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	A	+	+	+	+	+	+	+	M	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma															X		X								
Thyroid gland	+	A	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic														X											
Follicular cell, adenoma, multiple																									
GENERAL BODY SYSTEM																									
None																									
GENITAL SYSTEM																									
Ovary	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, spleen													X												
Lymphoma malignant lymphocytic														X											
Sarcoma											X														
Sarcoma stromal									X																
Vagina		A																							

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
CARCASS ID	444	445	452	453	454	455	461	462	463	464	465	472	473	474	475	483	484	485	494	495	501	502	503	504	505	TOTAL TISSUES TUMORS
ALIMENTARY SYSTEM																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant lymphocytic																										1
Gallbladder	+	M	+	+	+	+	+	M	+	M	+	+	+	+	M	+	+	+	M	+	M	+	+	+	+	35
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ileum, Peyer's patch, lymphoma malignant lymphocytic									X																	3
Jejunum, Peyer's patch, lymphoma malignant mixed																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma, metastatic spleen																										1
Hepatocellular carcinoma													X												X	4
Hepatocellular adenoma																										1
Lymphoma malignant lymphocytic									X																	2
Lymphoma malignant mixed																										3
Mesentery									+																	8
Lymphoma malignant lymphocytic									X																	2
Sarcoma stromal, metastatic, focal																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																										1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymphoma malignant lymphocytic																										1
CARDIOVASCULAR SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant lymphocytic																										1
Pheochromocytoma benign																										1
Cortex, lymphoma malignant mixed																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																										2
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	46
Pars distalis, adenoma	X																		X	X		X				6
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymphoma malignant lymphocytic																										1
Follicular cell, adenoma, multiple																						X				1
GENERAL BODY SYSTEM																										
None																										
GENITAL SYSTEM																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma, metastatic, spleen																										1
Lymphoma malignant lymphocytic																										1
Sarcoma																										1
Sarcoma stromal																										1
Vagina																										

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

WEEKS ON STUDY	0 6 6	0 8 4	0 8 6	0 8 8	0 9 1	0 9 2	0 9 2	0 9 8	1 0 0	1 0 0	1 0 0	1 0 0	1 0 4	1 0 4	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5
CARCASS ID	4 3 1	4 1 1	4 1 2	4 8 1	4 9 1	4 3 2	4 9 2	4 8 2	4 4 1	4 2 1	4 7 1	4 5 1	4 3 5	4 9 3	4 3 4	4 1 3	4 1 4	4 1 5	4 2 2	4 2 3	4 2 4	4 2 5	4 3 3	4 4 2	4 4 3
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, spleen													X												
Lymphoma malignant mixed						X																			
Lymph node	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skeletal muscle																									
Axillary, lymphoma malignant mixed						X																			
Bronchial, lymphoma malignant lymphocytic														X											
Bronchial, lymphoma malignant mixed	X					X																			
Inguinal, lymphoma malignant mixed						X																			
Mandibular, lymphoma malignant lymphocytic														X											
Mandibular, lymphoma malignant mixed						X						X													
Mediastinal, lymphoma malignant lymphocytic														X											
Mediastinal, lymphoma malignant mixed	X											X													
Mesenteric, lymphoma malignant lymphocytic														X				X							
Mesenteric, lymphoma malignant mixed	X					X						X													
Pancreatic, lymphoma malignant mixed	X					X																			
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma													X												
Lymphoma malignant lymphocytic																		X							
Lymphoma malignant mixed	X					X						X													
Thymus	M	A	M	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	M	+	
Lymphoma malignant lymphocytic														X											
INTEGUMENTARY SYSTEM																									
Mammary gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Lymphoma malignant lymphocytic														X											
Skin	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma																									
Subcutaneous tissue, lymphoma malignant lymphocytic														X											
Tail, papilloma squamous																									
MUSCULOSKELETAL SYSTEM																									
Bone	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Sarcoma																									
NERVOUS SYSTEM																									
Brain	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RESPIRATORY SYSTEM																									
Lung	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Lymphoma malignant lymphocytic																		X							
Lymphoma malignant mixed						X																			
Sarcoma, metastatic, skeletal muscle																									
Mediastinum, lymphoma malignant lymphocytic														X											
Nose	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	A	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSES SYSTEM																									
None																									
URINARY SYSTEM																									
Kidney	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic														X				X							
Lymphoma malignant mixed						X																			
Urinary bladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																		X							

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	TOTAL
CARCASS ID	444	445	452	453	454	455	461	462	463	464	465	472	473	474	475	483	484	485	494	495	501	502	503	504	505	TISSUES TUMORS
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma, metastatic, spleen																										1
Lymphoma malignant mixed																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma, metastatic, skeletal muscle			X																							1
Axillary, lymphoma malignant mixed																										1
Bronchial, lymphoma malignant lymphocytic																										1
Bronchial, lymphoma malignant mixed																										2
Inguinal, lymphoma malignant mixed																										1
Mandibular, lymphoma malignant lymphocytic									X																	2
Mandibular, lymphoma malig mixed																										2
Mediastinal, lymphoma malignant lymphocytic									X																	2
Mediastinal, lymphoma malig mixed																										2
Mesenteric, lymphoma malignant lymphocytic									X																	3
Mesenteric, lymphoma malignant mixed																										3
Pancreatic, lymphoma malignant mixed																										2
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Lymphoma malignant lymphocytic									X																	2
Lymphoma malignant mixed																										3
Thymus	+	M	+	+	+	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	40
Lymphoma malignant lymphocytic																										1
INTEGUMENTARY SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma															X			X								2
Lymphoma malignant lymphocytic																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Subcutaneous tissue, fibrosarcoma										X																1
Subcutaneous tissue, lymphoma malignant lymphocytic																										1
Tail, papilloma squamous									X																	1
MUSCULOSKELETAL SYSTEM																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skeletal muscle			+																							1
Sarcoma			X																							1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
RESPIRATORY SYSTEM																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma						X			X						X											3
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed																										1
Sarcoma, metastatic, skeletal muscle			X																							1
Mediastinum, lymphoma malignant lymphocytic																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SPECIAL SENSES SYSTEM																										
None																										
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant lymphocytic																										2
Lymphoma malignant mixed																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant lymphocytic																										1

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Control	500 ppm	1,000 ppm
Harderian Gland: Adenoma			
Overall Rates (a)	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted Rates (b)	0.0%	11.0%	0.0%
Terminal Rates (c)	0/39 (0%)	3/35 (9%)	0/36 (0%)
Day of First Observation		709	
Life Table Tests (d)	P=0.595	P=0.052	(e)
Logistic Regression Tests (d)	P=0.606	P=0.054	(e)
Cochran-Armitage Trend Test (d)	P=0.622		
Fisher Exact Test (d)		P=0.059	(e)
Harderian Gland: Adenoma or Carcinoma			
Overall Rates (a)	0/50 (0%)	5/50 (10%)	0/50 (0%)
Adjusted Rates (b)	0.0%	13.8%	0.0%
Terminal Rates (c)	0/39 (0%)	4/35 (11%)	0/36 (0%)
Day of First Observation		709	
Life Table Tests (d)	P=0.579	P=0.026	(e)
Logistic Regression Tests (d)	P=0.591	P=0.027	(e)
Cochran-Armitage Trend Test (d)	P=0.610		
Fisher Exact Test (d)		P=0.028	(e)
Liver: Hepatocellular Adenoma			
Overall Rates (a)	5/50 (10%)	3/50 (6%)	1/49 (2%)
Adjusted Rates (b)	12.8%	8.1%	2.8%
Terminal Rates (c)	5/39 (13%)	2/35 (6%)	1/36 (3%)
Day of First Observation	729	693	729
Life Table Tests (d)	P=0.087N	P=0.415N	P=0.121N
Logistic Regression Tests (d)	P=0.080N	P=0.397N	P=0.121N
Cochran-Armitage Trend Test (d)	P=0.073N		
Fisher Exact Test (d)		P=0.357N	P=0.107N
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	4/49 (8%)
Adjusted Rates (b)	7.4%	2.1%	11.1%
Terminal Rates (c)	2/39 (5%)	0/35 (0%)	4/36 (11%)
Day of First Observation	695	581	729
Life Table Tests (d)	P=0.385	P=0.338N	P=0.461
Logistic Regression Tests (d)	P=0.405	P=0.251N	P=0.474
Cochran-Armitage Trend Test (d)	P=0.402		
Fisher Exact Test (d)		P=0.309N	P=0.489
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	8/50 (16%)	4/50 (8%)	5/49 (10%)
Adjusted Rates (b)	19.9%	10.0%	13.9%
Terminal Rates (c)	7/39 (18%)	2/35 (6%)	5/36 (14%)
Day of First Observation	695	581	729
Life Table Tests (d)	P=0.258N	P=0.233N	P=0.326N
Logistic Regression Tests (d)	P=0.234N	P=0.182N	P=0.308N
Cochran-Armitage Trend Test (d)	P=0.225N		
Fisher Exact Test (d)		P=0.178N	P=0.290N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	3/50 (6%)	5/50 (10%)	3/49 (6%)
Adjusted Rates (b)	7.7%	13.5%	8.3%
Terminal Rates (c)	3/39 (8%)	4/35 (11%)	3/36 (8%)
Day of First Observation	729	667	729
Life Table Tests (d)	P=0.533	P=0.303	P=0.626
Logistic Regression Tests (d)	P=0.550	P=0.324	P=0.626
Cochran-Armitage Trend Test (d)	P=0.565		
Fisher Exact Test (d)		P=0.357	P=0.651

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Control	500 ppm	1,000 ppm
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	6/50 (12%)	3/49 (6%)
Adjusted Rates (b)	9.8%	16.3%	8.3%
Terminal Rates (c)	3/39 (8%)	5/35 (14%)	3/36 (8%)
Day of First Observation	693	667	729
Life Table Tests (d)	P=0.474N	P=0.312	P=0.539N
Logistic Regression Tests (d)	P=0.457N	P=0.338	P=0.526N
Cochran-Armitage Trend Test (d)	P=0.442N		
Fisher Exact Test (d)		P=0.370	P=0.511N
Pituitary Gland/Pars Distalis: Adenoma			
Overall Rates (a)	4/49 (8%)	4/44 (9%)	6/46 (13%)
Adjusted Rates (b)	10.5%	11.4%	17.1%
Terminal Rates (c)	4/38 (11%)	3/33 (9%)	6/35 (17%)
Day of First Observation	729	693	729
Life Table Tests (d)	P=0.261	P=0.569	P=0.317
Logistic Regression Tests (d)	P=0.261	P=0.558	P=0.317
Cochran-Armitage Trend Test (d)	P=0.269		
Fisher Exact Test (d)		P=0.581	P=0.330
Pituitary Gland/Pars Distalis: Adenoma or Carcinoma			
Overall Rates (a)	4/49 (8%)	5/44 (11%)	6/46 (13%)
Adjusted Rates (b)	10.5%	13.4%	17.1%
Terminal Rates (c)	4/38 (11%)	3/33 (9%)	6/35 (17%)
Day of First Observation	729	666	729
Life Table Tests (d)	P=0.268	P=0.427	P=0.317
Logistic Regression Tests (d)	P=0.264	P=0.415	P=0.317
Cochran-Armitage Trend Test (d)	P=0.273		
Fisher Exact Test (d)		P=0.431	P=0.330
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	16/50 (32%)	8/50 (16%)	7/50 (14%)
Adjusted Rates (b)	34.5%	20.5%	16.7%
Terminal Rates (c)	9/39 (23%)	5/35 (14%)	3/36 (8%)
Day of First Observation	630	440	459
Life Table Tests (d)	P=0.037N	P=0.100N	P=0.056N
Logistic Regression Tests (d)	P=0.012N	P=0.038N	P=0.018N
Cochran-Armitage Trend Test (d)	P=0.018N		
Fisher Exact Test (d)		P=0.050N	P=0.028N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors 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. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) No P value is reported because no tumors were observed in the 1,000-ppm and control groups.

TABLE D4a. HISTORICAL INCIDENCE OF HARDERIAN GLAND TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
HC Blue No. 2	0/50	0/50	0/50
C.I. Disperse Blue 1	1/50	0/50	1/50
D-Mannitol	0/48	0/48	0/48
Ziram	0/50	0/50	0/50
Eugenol	(b) 2/50	0/50	(b) 2/50
Propyl gallate	1/50	0/50	1/50
Zearalenone	1/50	0/50	1/50
HC Blue No. 1	2/50	0/50	2/50
Stannous chloride	3/50	0/50	3/50
TOTAL	(b) 10/448 (2.2%)	0/448 (0.0%)	(b) 10/448 (2.2%)
SD (c)	2.11%	0.00%	2.11%
Range (d)			
High	3/50	0/50	3/50
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	(e) 41/2,040 (2.0%)	(f) 7/2,040 (0.3%)	(e,f) 48/2,040 (2.4%)
SD (c)	2.06%	0.88%	2.19%
Range (d)			
High	4/50	2/50	4/50
Low	0/50	0/50	0/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Includes one cystadenoma, NOS
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes three papillary adenomas, one cystadenoma, NOS, and two papillary cystadenomas, NOS
(f) Includes one adenocarcinoma, NOS, two papillary adenocarcinomas, and one papillary cystadenocarcinoma, NOS

TABLE D4b. HISTORICAL INCIDENCE OF HEMATOPOIETIC SYSTEM TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls	
	Lymphoma	Lymphoma or Leukemia
Historical Incidence at Southern Research Institute		
HC Blue No. 2	12/50	12/50
C.I. Disperse Blue 1	17/50	17/50
D-Mannitol	14/48	14/48
Ziram	6/50	11/50
Eugenol	12/50	13/50
Propyl gallate	8/50	9/50
Zearalenone	15/50	15/50
HC Blue No. 1	6/50	7/50
Stannous chloride	5/50	6/50
TOTAL	95/448 (21.2%)	104/448 (23.2%)
SD (b)	8.96%	7.46%
Range (c)		
High	17/50	17/50
Low	5/50	6/50
Overall Historical Incidence		
TOTAL	617/2,040 (30.2%)	636/2,040 (31.2%)
SD (b)	13.32%	12.83%
Range (c)		
High	37/50	38/50
Low	5/50	6/50

(a) Data as of April 29, 1987, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

	Untreated Control	Low Dose	High Dose
Animals initially in study	50	50	50
Animals removed	50	50	50
Animals examined histopathologically	50	50	50
ALIMENTARY SYSTEM			
Intestine small	(49)	(45)	(48)
Duodenum, jejunum, autolysis	1 (2%)		
Ileum, amyloid deposition	1 (2%)		
Ileum, Peyer's patch, foreign body		1 (2%)	
Ileum, Peyer's patch, hyperplasia, lymphoid	1 (2%)	1 (2%)	
Ileum, Peyer's patch, inflammation, suppurative		1 (2%)	
Jejunum, Peyer's patch, hyperplasia, lymphoid	1 (2%)		1 (2%)
Wall, inflammation, suppurative	2 (4%)		
Liver	(50)	(50)	(49)
Angiectasis		2 (4%)	
Hematopoietic cell proliferation	9 (18%)	4 (8%)	6 (12%)
Hemorrhage	1 (2%)		
Infiltration cellular, lymphocytic	3 (6%)	5 (10%)	4 (8%)
Inflammation, granulomatous			1 (2%)
Inflammation, suppurative	2 (4%)	2 (4%)	1 (2%)
Mitotic alteration		1 (2%)	
Necrosis, focal	2 (4%)		1 (2%)
Necrosis, multifocal	1 (2%)		
Nuclear alteration		1 (2%)	
Thrombus		1 (2%)	
Hepatocyte, atrophy, focal		1 (2%)	
Mesentery	(11)	(3)	(8)
Inflammation, suppurative	4 (36%)	1 (33%)	6 (75%)
Fat, inflammation, chronic	1 (9%)		
Fat, necrosis, focal	3 (27%)	2 (67%)	
Pancreas	(49)	(48)	(47)
Infiltration cellular, lymphocytic		1 (2%)	
Inflammation, suppurative	1 (2%)	2 (4%)	
Acinus, atrophy, focal	1 (2%)		1 (2%)
Acinus, atrophy, multifocal		1 (2%)	1 (2%)
Acinus, hyperplasia		1 (2%)	
Duct, cyst	2 (4%)	1 (2%)	
Duct, inflammation, suppurative	1 (2%)		
Stomach	(49)	(48)	(47)
Forestomach, diverticulum	1 (2%)		
Forestomach, foreign body			1 (2%)
Forestomach, hyperkeratosis, focal	1 (2%)		
Forestomach, inflammation, granulomatous			1 (2%)
Forestomach, inflammation, suppurative	2 (4%)	2 (4%)	
Forestomach, ulcer		1 (2%)	1 (2%)
Glandular, hyperplasia, focal			1 (2%)
Tooth		(1)	
Dysplasia		1 (100%)	
CARDIOVASCULAR SYSTEM			
Heart	(50)	(50)	(49)
Inflammation, suppurative, focal	1 (2%)		

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
Adrenal gland	(49)	(49)	(49)
Capsule, degeneration, fatty	1 (2%)		
Cortex, degeneration, fatty, focal	1 (2%)	2 (4%)	2 (4%)
Cortex, hyperplasia, diffuse			1 (2%)
Medulla, degeneration, fatty		1 (2%)	
Medulla, hyperplasia, focal	1 (2%)		
Spindle cell, hyperplasia, multifocal		1 (2%)	
Pituitary gland	(49)	(44)	(46)
Pars distalis, angiectasis	8 (16%)	3 (7%)	6 (13%)
Pars distalis, cyst	2 (4%)		1 (2%)
Pars distalis, hyperplasia, focal	5 (10%)	8 (18%)	5 (11%)
Thyroid gland	(50)	(50)	(48)
Cyst	1 (2%)		1 (2%)
Degeneration, cystic	6 (12%)	5 (10%)	7 (15%)
Hyperplasia, cystic	4 (8%)	1 (2%)	3 (6%)
Inflammation, suppurative			1 (2%)
Follicular cell, hyperplasia, diffuse	1 (2%)	1 (2%)	
Follicular cell, hyperplasia, focal	3 (6%)		3 (6%)
Follicular cell, hyperplasia, multifocal		1 (2%)	4 (8%)
GENERAL BODY SYSTEM			
None			
GENITAL SYSTEM			
Ovary	(48)	(45)	(48)
Cyst	12 (25%)	20 (44%)	16 (33%)
Inflammation, suppurative	9 (19%)	6 (13%)	6 (13%)
Uterus	(50)	(50)	(49)
Angiectasis			1 (2%)
Hydrometria			1 (2%)
Hyperplasia, cystic	47 (94%)	48 (96%)	44 (90%)
Hyperplasia, glandular			1 (2%)
Inflammation, suppurative	8 (16%)	9 (18%)	11 (22%)
Wall, thrombus		1 (2%)	
HEMATOPOIETIC SYSTEM			
Bone marrow	(48)	(48)	(49)
Myelofibrosis	2 (4%)	3 (6%)	2 (4%)
Myeloid cell, hyperplasia	6 (13%)	3 (6%)	5 (10%)
Lymph node	(50)	(50)	(49)
Iliac, autolysis			1 (2%)
Iliac, hyperplasia, lymphoid		1 (2%)	
Lumbar, angiectasis	1 (2%)		
Lumbar, hyperplasia, lymphoid		1 (2%)	1 (2%)
Mandibular, hyperplasia, lymphoid		1 (2%)	3 (6%)
Mediastinal, hyperplasia, lymphoid	2 (4%)		1 (2%)
Mediastinal, inflammation, suppurative		1 (2%)	2 (4%)
Mesenteric, angiectasis	4 (8%)	1 (2%)	2 (4%)
Mesenteric, hematopoietic cell proliferation	1 (2%)	2 (4%)	
Mesenteric, hyperplasia, lymphoid	3 (6%)	3 (6%)	4 (8%)
Pancreatic, hyperplasia, lymphoid			1 (2%)
Renal, hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)
Spleen	(49)	(49)	(49)
Atrophy		1 (2%)	
Hematopoietic cell proliferation	10 (20%)	7 (14%)	12 (24%)
Hyperplasia, lymphoid	4 (8%)	5 (10%)	6 (12%)
Necrosis, focal	1 (2%)		
Pigmentation		3 (6%)	1 (2%)

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
Thymus	(48)	(48)	(40)
Hyperplasia, lymphoid		1 (2%)	1 (3%)
Mediastinum, inflammation, suppurative	1 (2%)		
INTEGUMENTARY SYSTEM			
Mammary gland	(46)	(47)	(49)
Hyperplasia, lobular		1 (2%)	
Inflammation, suppurative		1 (2%)	
Duct, ectasia		1 (2%)	3 (6%)
Skin	(50)	(50)	(49)
Fibrosis, focal			1 (2%)
Infiltration cellular, mast cell		1 (2%)	
MUSCULOSKELETAL SYSTEM			
Bone	(50)	(50)	(49)
Fibrous osteodystrophy	1 (2%)	2 (4%)	2 (4%)
Skeletal muscle	(1)	(1)	(1)
Mineralization		1 (100%)	
NERVOUS SYSTEM			
Brain	(50)	(48)	(49)
Compression			1 (2%)
Corpora amylacea	8 (16%)	6 (13%)	13 (27%)
Hemorrhage, multifocal	1 (2%)		
Meninges, infiltration cellular, lymphocytic		1 (2%))	
RESPIRATORY SYSTEM			
Lung	(50)	(50)	(49)
Hemorrhage	2 (4%)		1 (2%)
Infiltration cellular, lymphocytic	3 (6%)	2 (4%)	5 (10%)
Inflammation, suppurative	2 (4%)		1 (2%)
Necrosis	1 (2%)		
Alveolar epithelium, hyperplasia, focal		2 (4%)	4 (8%)
Mediastinum, inflammation, suppurative	1 (2%)	3 (6%)	2 (4%)
SPECIAL SENSES SYSTEM			
Harderian gland	(1)	(5)	
Hyperplasia	1 (100%)		

TABLE D5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G (Continued)

	Untreated Control	Low Dose	High Dose
URINARY SYSTEM			
Kidney	(50)	(50)	(49)
Atrophy		1 (2%)	
Casts protein			1 (2%)
Cyst		1 (2%)	
Glomerulosclerosis			2 (4%)
Hydronephrosis		1 (2%)	
Infiltration cellular, lymphocytic	21 (42%)	29 (58%)	28 (57%)
Inflammation, chronic		1 (2%)	
Inflammation, granulomatous, focal			1 (2%)
Metaplasia, osseous		1 (2%)	1 (2%)
Nephropathy, chronic	3 (6%)	5 (10%)	3 (6%)
Glomerulus, amyloid deposition		1 (2%)	1 (2%)
Interstitial tissue, mineralization		2 (4%)	1 (2%)
Renal tubule, dilatation		1 (2%)	1 (2%)
Urinary bladder	(48)	(47)	(49)
Infiltration cellular, lymphocytic	1 (2%)	1 (2%)	2 (4%)
Inflammation, granulomatous			1 (2%)

APPENDIX E

SENTINEL ANIMAL PROGRAM

APPENDIX E. 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 viral serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are both 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.

Fifteen B6C3F$_1$ mice and 15 F344/N rats of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Data from animals surviving 24 months were collected from 5/50 randomly selected control animals of each sex and species. The blood from each animal was collected and clotted, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the viral antibody titers. The following tests were performed:

	Hemagglutination Inhibition	Complement Fixation	ELISA
Mice	PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Ectro (infectious ectromelia) Sendai	M. Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus) MHV (6 mo)	MHV (mouse hepatitis virus) (12,18,24 mo)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai	RCV (rat coronavirus)	

Results

Results are presented in Table E1.

TABLE E1. MURINE VIRUS ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF RHODAMINE 6G (a)

Interval (months)	Number of Animals	Positive Serologic Reaction for
RATS		
6	--	None positive
12	--	None positive
18	--	None positive
24	2/10	KRV
MICE		
6	--	None positive
12	2/10	LCM
(b) 14	--	None positive
18	--	None positive
24	--	None positive

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing and from the control animals just before they were killed; samples were sent to Microbiological Associates (Bethesda, MD) for determination of antibody titers.
(b) Blood samples were taken from sentinel mice at 14 months by orbital bleeding for a special screening for LCM by complement fixation and an immunofluorescence assay.

APPENDIX F

FEED AND COMPOUND CONSUMPTION BY RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF RHODAMINE 6G

TABLE F1. FEED AND COMPOUND CONSUMPTION BY MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

Week	Control		Low Dose			High Dose		
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
5	18	272	17	271	8	18	278	16
9	18	326	16	328	6	17	328	13
16	16	374	16	379	5	16	378	11
20	18	385	18	394	5	17	393	11
24	18	394	17	402	5	17	401	11
29	19	422	19	429	5	19	424	11
34	19	431	19	445	5	17	440	10
39	19	446	20	460	5	24	453	13
43	17	452	18	468	5	17	460	9
47	18	456	18	471	5	16	467	9
51	17	465	18	484	4	17	476	9
56	17	472	17	491	4	17	481	9
60	17	476	18	495	4	18	486	9
63	17	481	17	504	4	17	492	9
67	16	482	17	502	4	16	493	8
72	17	482	17	501	4	17	496	9
77	16	456	17	499	4	17	497	9
81	11	468	15	497	4	16	496	8
86	16	450	18	479	5	17	484	9
91	15	464	17	467	4	17	480	9
95	16	445	17	458	4	17	467	9
99	15	429	17	451	5	17	458	9
104	18	435	18	437	5	18	446	10
Mean	16.9	433	17.4	448	5	17.3	447	10
SD (c)	1.7		1.1		1	1.6		2
CV (d)	10.1		6.3		20.0	9.2		20.0

(a) Grams of feed removed from the feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of rhodamine 6G consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

TABLE F2. FEED AND COMPOUND CONSUMPTION BY FEMALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

Week	Control		Low Dose			High Dose		
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
5	13	172	12	175	8	12	172	17
9	12	193	11	197	7	11	194	14
16	12	213	12	217	7	12	211	14
20	12	219	11	226	6	11	217	13
24	13	226	12	232	6	12	224	13
29	13	239	12	244	6	13	236	14
34	13	243	12	252	6	12	244	12
39	12	251	12	252	6	13	252	13
43	12	256	11	263	5	13	257	13
47	13	264	12	268	5	13	262	12
51	13	274	13	280	6	13	273	12
56	11	283	12	290	5	12	283	11
60	13	296	12	301	5	14	292	12
63	13	305	13	314	5	14	305	11
67	13	311	12	321	4	13	313	10
72	13	322	13	333	5	14	322	11
77	12	320	12	323	4	13	329	10
81	8	335	7	345	2	12	338	9
86	14	337	14	348	5	13	338	10
91	14	348	14	356	5	13	344	9
95	14	349	13	360	4	14	348	10
99	14	357	14	365	5	14	348	10
104	14	352	13	369	4	14	347	10
Mean	12.7	281	12.1	288	5	12.8	280	12
SD (c)	1.3		1.4		1	0.9		2
CV (d)	10.2		11.6		20.0	7.0		16.7

(a) Grams of feed removed from the feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of rhodamine 6G consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

TABLE F3. FEED AND COMPOUND CONSUMPTION BY MALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

Week	Control		Low Dose			High Dose		
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
5	8.1	30.8	8.1	29.8	272	8.0	29.5	542
9	7.4	33.5	7.6	31.8	239	7.1	31.0	458
17	7.5	36.5	7.3	34.6	211	6.9	33.4	413
21	5.8	37.0	7.0	34.9	201	7.0	33.8	414
25	7.3	37.7	7.3	36.4	201	7.0	34.2	409
29	7.0	38.7	7.2	36.9	195	7.0	34.9	401
35	7.9	39.8	7.3	37.8	193	6.9	35.3	391
40	7.0	40.0	7.6	38.4	198	7.3	35.7	409
43	8.1	39.9	7.5	38.1	197	7.6	35.4	429
48	8.6	40.7	8.2	39.0	210	7.8	35.9	435
52	7.9	41.0	7.3	39.2	186	6.6	35.6	371
57	8.0	40.4	7.2	38.7	186	6.8	35.4	384
61	8.6	40.9	8.2	39.0	210	7.8	35.4	441
64	8.6	40.8	8.0	38.5	208	8.1	35.9	451
68	8.4	41.2	8.0	38.5	208	8.0	35.8	447
73	9.6	41.1	7.7	38.5	200	7.5	35.5	423
78	7.7	40.1	7.6	38.6	197	7.8	35.8	436
82	7.3	40.6	6.9	38.0	182	7.8	35.2	443
87	7.7	38.8	7.9	36.9	214	8.0	34.5	464
91	7.7	39.3	8.2	37.7	218	8.1	34.7	467
95	7.8	38.4	9.3	35.9	259	8.0	34.4	465
99	8.6	38.5	9.4	36.7	256	7.8	34.2	456
104	8.8	37.2	9.5	36.3	262	8.4	34.7	484
Mean	7.9	38.8	7.8	37.0	213	7.5	34.6	436
SD (c)	0.8		0.7		26	0.5		37
CV (d)	10.1		9.0		12.2	6.7		8.5

(a) Grams of feed removed from the feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of rhodamine 6G consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

TABLE F4. FEED AND COMPOUND CONSUMPTION BY FEMALE MICE IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G

Week	Control		Low Dose			High Dose		
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)	Grams Feed/ Day (a)	Body Weight (grams)	Dose/ Day (b)
5	7.8	22.0	8.1	21.7	187	7.9	22.6	350
9	7.3	24.5	7.3	23.4	156	6.7	23.5	285
17	7.2	26.4	7.4	25.7	144	7.1	25.5	278
21	7.0	27.9	6.8	26.2	130	7.0	26.6	263
25	7.0	28.0	6.9	27.4	126	7.8	27.1	288
29	6.9	30.1	7.1	28.8	123	7.5	28.3	265
35	7.2	31.0	7.0	29.2	120	7.4	28.8	257
40	6.1	32.3	7.2	29.1	124	7.2	30.4	237
43	8.2	32.9	7.6	29.8	128	7.3	30.6	239
48	9.0	34.2	7.8	31.0	126	8.1	31.3	259
52	7.3	34.5	7.3	32.3	113	7.1	32.5	218
57	8.6	35.2	7.2	32.8	110	7.2	32.6	221
61	8.4	36.9	8.1	32.8	123	9.0	32.7	275
64	8.1	37.6	8.0	33.6	119	7.9	33.9	233
68	8.5	37.7	7.6	33.8	112	8.4	33.4	251
73	8.9	39.8	7.5	35.5	106	7.4	34.1	217
78	8.4	39.9	7.3	34.6	105	7.7	34.3	224
82	8.0	38.4	7.3	34.8	105	7.2	33.7	214
87	7.4	37.0	7.5	34.1	110	7.7	33.4	231
91	7.5	38.2	7.9	35.0	113	7.9	34.4	230
95	7.5	38.2	8.3	34.7	120	8.7	34.5	252
99	8.1	39.5	9.1	35.3	129	8.3	35.2	236
104	8.1	40.5	9.7	35.3	137	8.8	36.1	244
Mean	7.8	34.0	7.7	31.2	125	7.7	31.1	251
SD (c)	0.7		0.7		18	0.6		31
CV (d)	9.0		9.1		14.4	7.8		12.4

(a) Grams of feed removed from the feeder per animal per day; not corrected for scatter.
(b) Estimated milligrams of rhodamine 6G consumed per day per kilogram of body weight
(c) Standard deviation
(d) Coefficient of variation = (standard deviation/mean) × 100

APPENDIX G

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

Meal Diet: December 1980 to January 1983

(Manufactured by Zeigler Bros., Inc., Gardners, PA)

TABLE G1. INGREDIENTS OF NIH 07 RAT AND MOUSE RATION (a)

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

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

TABLE G2. 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 G3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION (a)

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Crude protein (percent by weight)	24.25 ± 1.04	22.6-26.3	24
Crude fat (percent by weight)	5.10 ± 0.44	4.4-6.0	24
Crude fiber (percent by weight)	3.38 ± 0.38	2.4-4.2	24
Ash (percent by weight)	6.59 ± 0.34	5.97-7.42	24
Amino Acids (percent of total diet)			
Arginine	1.323 ± 0.830	1.21-1.39	4
Cystine	0.310 ± 0.099	0.218-0.400	4
Glycine	1.155 ± 0.069	1.06-1.21	4
Histidine	0.572 ± 0.030	0.530-0.603	4
Isoleucine	0.910 ± 0.033	0.881-0.944	4
Leucine	1.949 ± 0.065	1.85-1.99	4
Lysine	1.275 ± 0.076	1.20-1.37	4
Methionine	0.422 ± 0.187	0.306-0.699	4
Phenylalanine	0.909 ± 0.167	0.665-1.04	4
Threonine	0.844 ± 0.029	0.824-0.886	4
Tryptophan	0.187	0.171-0.211	3
Tyrosine	0.631 ± 0.094	0.566-0.769	4
Valine	1.11 ± 0.050	1.05-1.17	4
Essential Fatty Acids (percent of total diet)			
Linoleic	2.44	2.37-2.52	3
Linolenic	0.274	0.256-0.308	3
Arachidonic	0.008		1
Vitamins			
Vitamin A (IU/kg)	11,188 ± 1,239	8,900-1,400	24
Vitamin D (IU/kg)	4,650	3,000-6,300	2
α-Tocopherol (ppm)	41.53 ± 7.52	31.1-48.9	4
Thiamine (ppm)	16.2 ± 2.30	12.0-21.0	(b) 23
Riboflavin (ppm)	7.5 ± 0.96	6.1-8.2	4
Niacin (ppm)	85.0 ± 14.2	65.0-97.0	4
Pantothenic acid (ppm)	29.3 ± 4.6	23.0-34.0	4
Pyridoxine (ppm)	7.6 ± 1.5	5.6-8.8	4
Folic acid (ppm)	2.8 ± 0.88	1.8-3.7	4
Biotin (ppm)	0.27 ± 0.05	0.21-0.32	4
Vitamin B_{12} (ppb)	21.0 ± 11.9	11.0-38.0	4
Choline (ppm)	3,302.0 ± 120.0	3,200.0-3,430.0	4
Minerals			
Calcium (percent)	1.23 ± 0.12	1.10-1.53	24
Phosphorus (percent)	0.97 ± 0.06	0.84-1.10	24
Potassium (percent)	0.862 ± 0.100	0.772-0.974	3
Chloride (percent)	0.546 ± 0.100	0.442-0.635	4
Sodium (percent)	0.311 ± 0.038	0.258-0.350	4
Magnesium (percent)	0.169 ± 0.133	0.151-0.181	4
Sulfur (percent)	0.316 ± 0.070	0.270-0.420	4
Iron (ppm)	447.0 ± 57.3	409.0-523.0	4
Manganese (ppm)	90.6 ± 8.20	81.7-95.5	4
Zinc (ppm)	53.6 ± 5.27	46.1-58.6	4
Copper (ppm)	10.77 ± 3.19	8.09-15.39	4
Iodine (ppm)	2.95 ± 1.05	1.52-3.82	4
Chromium (ppm)	1.81 ± 0.28	1.44-2.09	4
Cobalt (ppm)	0.68 ± 0.14	0.49-0.80	4

(a) One to four lots of feed analyzed for nutrients reported in this table were manufactured during 1983-85.
(b) One lot (7/22/81) not analyzed for thiamine

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminants	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.44 ± 0.14	0.21-0.93	24
Cadmium (ppm) (a)	<0.1		24
Lead (ppm)	1.03 ± 0.75	0.27-2.93	24
Mercury (ppm) (a)	< 0.05		24
Selenium (ppm)	0.27 ± 0.05	0.16-0.40	24
Aflatoxins (ppb) (a,b)	<10	<5.0-10.0	24
Nitrate nitrogen (ppm) (c)	9.35 ± 4.35	0.6-18.0	24
Nitrite nitrogen (ppm) (c)	1.97 ± 1.28	0.4-5.3	24
BHA (ppm) (d)	5.83 ± 5.12	0.4-20.0	24
BHT (ppm) (d)	3.42 ± 2.57	<1.0-13.0	24
Aerobic plate count (CFU/g) (e)	105,438 ± 75,797	7,000-300,000	24
Coliform (MPN/g) (f)	1,046 ± 973	<3-2,400	24
E. coli (MPN/g) (g)	8.0 ± 7.91	<3-23	23
E. coli (MPN/g) (h)	13.92 ± 30.0	<3-150	24
Total nitrosamines (ppb) (i,j)	5.13 ± 4.47	<1.2-18.8	22
Total nitrosamines (ppb) (i,k)	13.11 ± 27.39	<1.2-101.6	24
N-Nitrosodimethylamine (ppb) (i,l)	3.82 ± 4.29	0.6-16.8	22
N-Nitrosodimethylamine (ppb) (i,m)	11.71 ± 27.03	0.6-99	24
N-Nitrosopyrrolidine (ppb)	1.21 ± 0.66	<0.3-2.4	24
Pesticides (ppm)			
α-BHC (a,n)	<0.01		24
β-BHC (a)	<0.02		24
γ-BHC-Lindane (a)	<0.01		24
δ-BHC (a)	<0.01		24
Heptachlor (a)	<0.01		24
Aldrin (a)	<0.01		24
Heptachlor epoxide (a)	<0.01		24
DDE (o)	<0.01	0.05 (7/14/81)	24
DDD (a)	<0.01		24
DDT (a)	<0.01		24
HCB (a)	<0.01		24
Mirex (a)	<0.01		24
Methoxychlor (p)	<0.05	0.13 (8/25/81); 0.6 (6/29/82)	24
Dieldrin (a)	<0.01		24
Endrin (a)	<0.01		24
Telodrin (a)	<0.01		24
Chlordane (a)	<0.05		24
Toxaphene (a)	<0.1		24
Estimated PCBs (a)	<0.2		24
Ronnel (a)	<0.01		24
Ethion (a)	<0.02		24
Trithion (a)	<0.05		24
Diazinon (a)	<0.1		24
Methyl parathion (a)	<0.02		24
Ethyl parathion (a)	<0.02		24
Malathion (q)	0.08 ± 0.05	<0.05-0.25	24
Endosulfan I (a)	<0.01		24
Endosulfan II (a)	<0.01		24
Endosulfan sulfate (a)	<0.03		24

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) All values were less than the detection limit, given in the table as the mean.
(b) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(c) Source of contamination: alfalfa, grains, and fish meal
(d) Source of contamination: soy oil and fish meal
(e) CFU = colony-forming unit
(f) MPN = most probable number
(g) Mean, standard deviation, and range exclude one high value of 150 obtained for the lot produced on 8/26/82.
(h) Mean, standard deviation, and range include the high value listed in footnote (g).
(i) All values were corrected for percent recovery.
(j) Mean, standard deviation, and range exclude two very high values of 101.6 and 100.3 ppb obtained for the lots produced on 1/26/81 and 4/27/81.
(k) Mean, standard deviation, and range include the high values listed in footnote (j).
(l) Mean, standard deviation, and range exclude two very high values of 97.9 and 99.0 ppb for lots produced on 1/26/81 and 4/27/81.
(m) Mean, standard deviation, and range include the very high values given in footnote (l).
(n) BHC = hexachlorocyclohexane or benzene hexachloride
(o) One observation was above the detection limit; the value and date it was obtained are listed under the range.
(p) Two observations were above the detection limit; the values and dates they were obtained are listed under the range.
(q) Eleven lots contained more than 0.05 ppm.

APPENDIX H

AUDIT SUMMARY

APPENDIX H. AUDIT SUMMARY

The pathology specimens, experimental data, study documents, and draft NTP Technical Report No. 364 for the 2-year studies of rhodamine 6G in rats and mice were audited for the National Institute of Environmental Health Sciences (NIEHS) at the National Toxicology Program (NTP) Archives by Program Resources, Inc., and Argus Research Laboratories. The audit included review of:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
(2) All inlife records including protocol, correspondence, animal husbandry, environmental conditions, dosing, external masses, mortality, animal identification, and serology.
(3) Feed consumption, body weight, and clinical observation data; all data were scanned before individual data for a random 10% sample of animals in each study group were reviewed in detail.
(4) All chemistry records.
(5) All postmortem records for individual animals concerning date of death, disposition code, condition code, tissue accountability, correlation of masses or clinical signs recorded at the last inlife observation with gross observations and microscopic diagnoses, and correlations between gross observations and microscopic diagnoses.
(6) All wet tissue bags for inventory and wet tissues from a random 20% sample of animals in each study group, plus other relevant cases, to evaluate the integrity of individual animal identity and to examine for untrimmed potential lesions.
(7) Blocks and slides of tissues from a random 20% sample of animals from each study group, plus animals with less than complete or correct identification.
(8) Necropsy record forms for data entry errors and all microscopic diagnosis updates for a random 10% sample of animals to verify their incorporation into final pathology tables.
(9) Correlation between the data, factual information, and procedures for the 2-year studies presented in the draft Technical Report and the records available at the NTP Archives.

Procedures and events during the exposure phase of the studies were documented adequately by the archival records, except for the randomization of animals to study groups and disposition of extra animals prior to start of studies. Examination of average group body weights at the start of the studies verified their uniform distribution across study groups. Review of data from the entire exposure phase indicated that animal care procedures were effective and consistent during the course of the studies. Records documented that doses were prepared, stored, analyzed, and administered to animals according to protocols. Recalculation of 24 group mean body weight values showed all to be correct. Of the masses noted in the inlife records, 167/177 in rats and 138/143 in mice correlated with necropsy observations. Survival records for all animals were reviewed and found to be correct, except for the date of death for one high dose female rat and reason for removal of one high dose male mouse; the corrected information is reported in the NTP Technical Report.

Individual animal identifiers (punched ears) were present and correct for 71/72 rats and 72/74 mice examined. One ear was correct for each of the remaining two mice and one rat examined, whereas punches in the second ear were in a wrong location or were unreadable; review of data trails for these animals provided evidence that the integrity of their individual animal identity had been preserved throughout the studies. The residual wet tissues contained four untrimmed potential lesions in rats and one in mice that involved nontarget organs. Gross observations made at necropsy correlated with microscopic diagnoses, except for four observations that involved nontarget organs.

Full details about these and other audit findings are presented in audit reports on file at the NIEHS. In conclusion, the data and factual information in the Technical Report for the 2-year feed studies of rhodamine 6G are supported by the records at the NTP Archives.

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