

NATIONAL TOXICOLOGY PROGRAM
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No. 364



TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

RHODAMINE 6G

(C.I. BASIC RED 1)

(CAS NO. 989-38-8)

IN F344/N RATS AND B6C3F₁ 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 B6C3F₁ 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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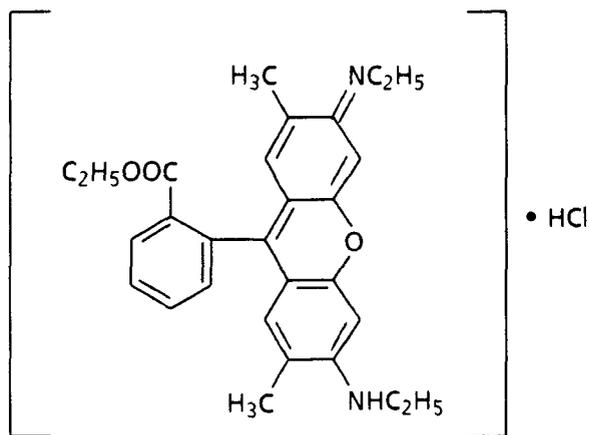
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RHODAMINE 6G

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

CAS No. 989-38-8

$C_{28}H_{30}N_2O_3 \cdot HCl$

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; Nyc 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 B6C3F₁ 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 B6C3F₁ mice administered 1,000 or 2,000 ppm rhodamine 6G in the diet. There was *no evidence of carcinogenic activity* for female B6C3F₁ 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 pheochromocy- tomas (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 <u>Salmonella</u> <u>Gene Mutation</u> Negative with and without S9 | <u>Mouse L5178Y/TK</u> <u>Tft Resistance</u> Positive without S9; negative with S9 | <u>CHO Cells in Vitro</u> <u>SCE</u> <u>Aberration</u> Negative without S9; Negative without S9; positive with 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

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

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

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

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

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

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

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.

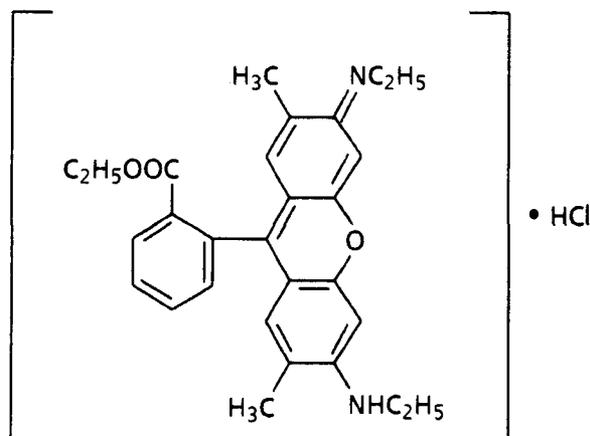
Dr. Gallo, the second principal reviewer, agreed with the conclusions. He speculated that chemical interaction with the epidermal growth factor receptor complex may have played a role in the induction of skin tumors in male rats. Rhodamine compounds are photoactive, and many photoactive compounds have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



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

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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; Nyc 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

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 (LC₅₀ = 0.67 × 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-c₁ 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

I. INTRODUCTION

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 VA₂-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
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II. MATERIALS AND METHODS

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 KC₁₈ 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 μ Bondapak C₁₈ 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

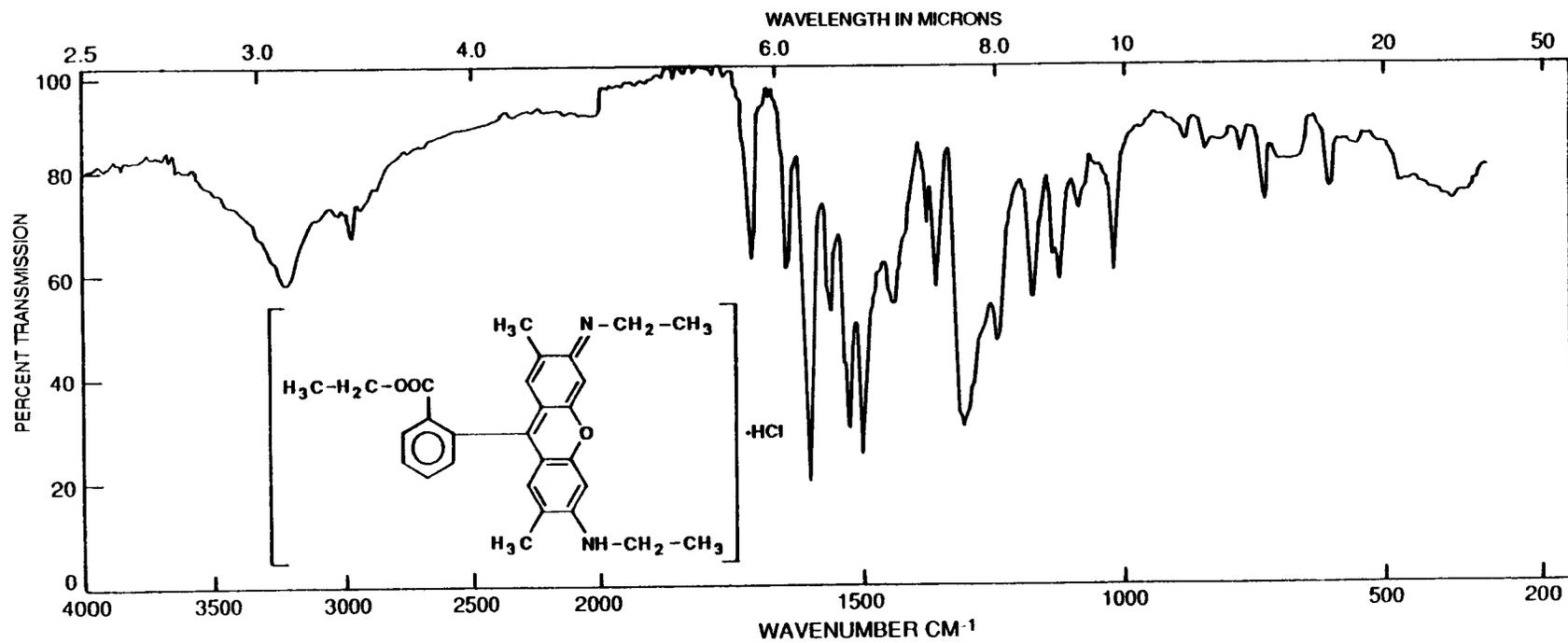


FIGURE 1. INFRARED ABSORPTION SPECTRUM OF RHODAMINE 6G (LOT NO. 14-6907)

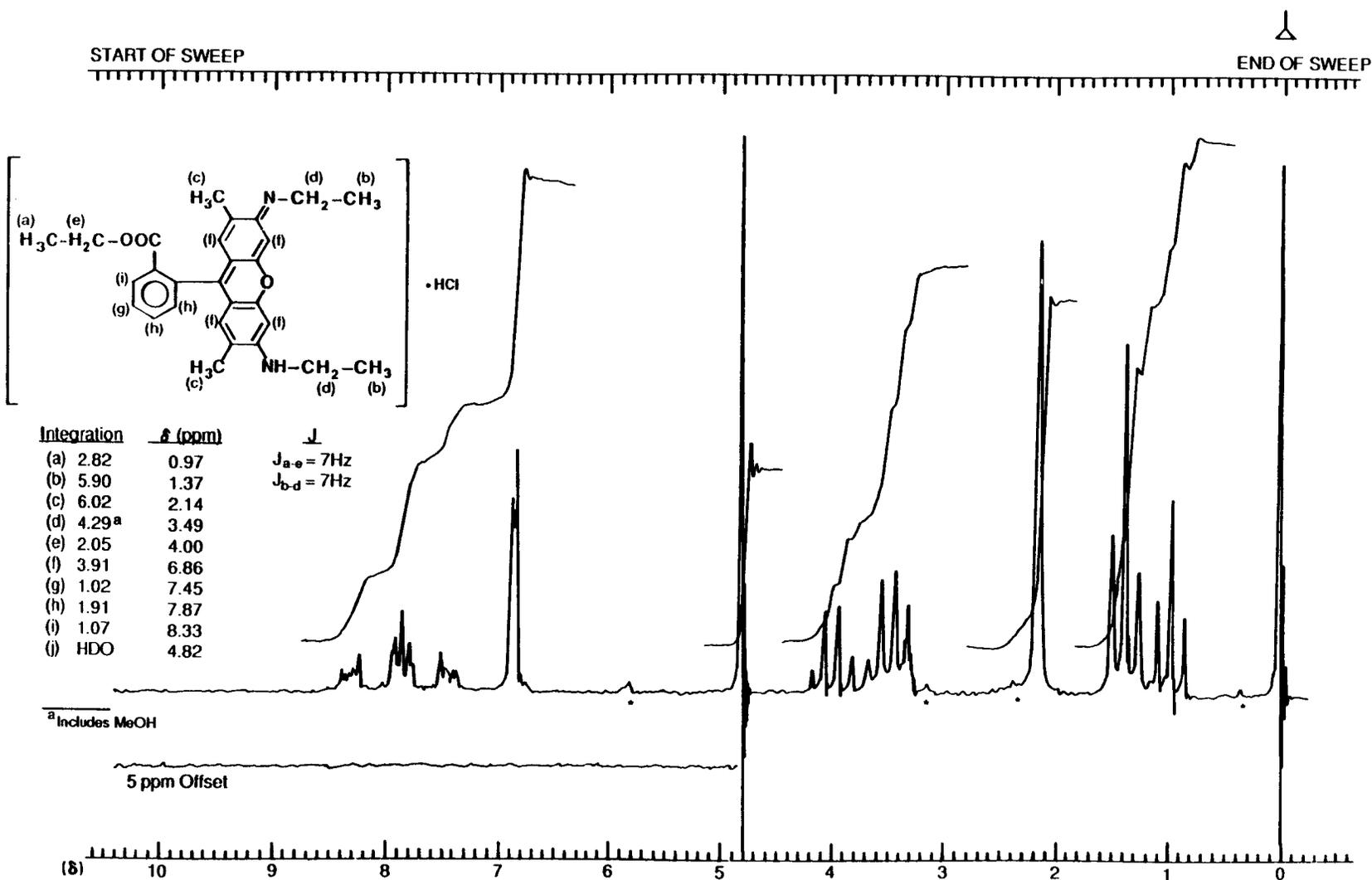


FIGURE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF RHODAMINE 6G (LOT NO. 14-6907)

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 C₁₈ 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 B6C3F₁ 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 B6C3F₁ 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 B6C3F₁ 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 (North-eastern 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 B6C3F₁ (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 B6C3F₁ 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 B6C3F₁ mice used in these studies. The influence of the potential genetic non-uniformity 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.

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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

II. MATERIALS AND METHODS

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

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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 non-selective 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.

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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

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III. RESULTS: RATS

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) LD₅₀ 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) LD₅₀ by Spearman-Kärber 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) | | | Final Weight Relative to Controls (percent) | Feed Consumption (d) | |
|---------------------|--------------|---------------------------|---------|------------|---|----------------------|--------|
| | | Initial (b) | Final | Change (c) | | 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) | | | Final Weight Relative to Controls (percent) | Feed Con- sumption (d) | |
|------------------------|-----------------|---------------------------|---------|------------|---|---------------------------|---------|
| | | Initial (b) | Final | Change (c) | | 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 |

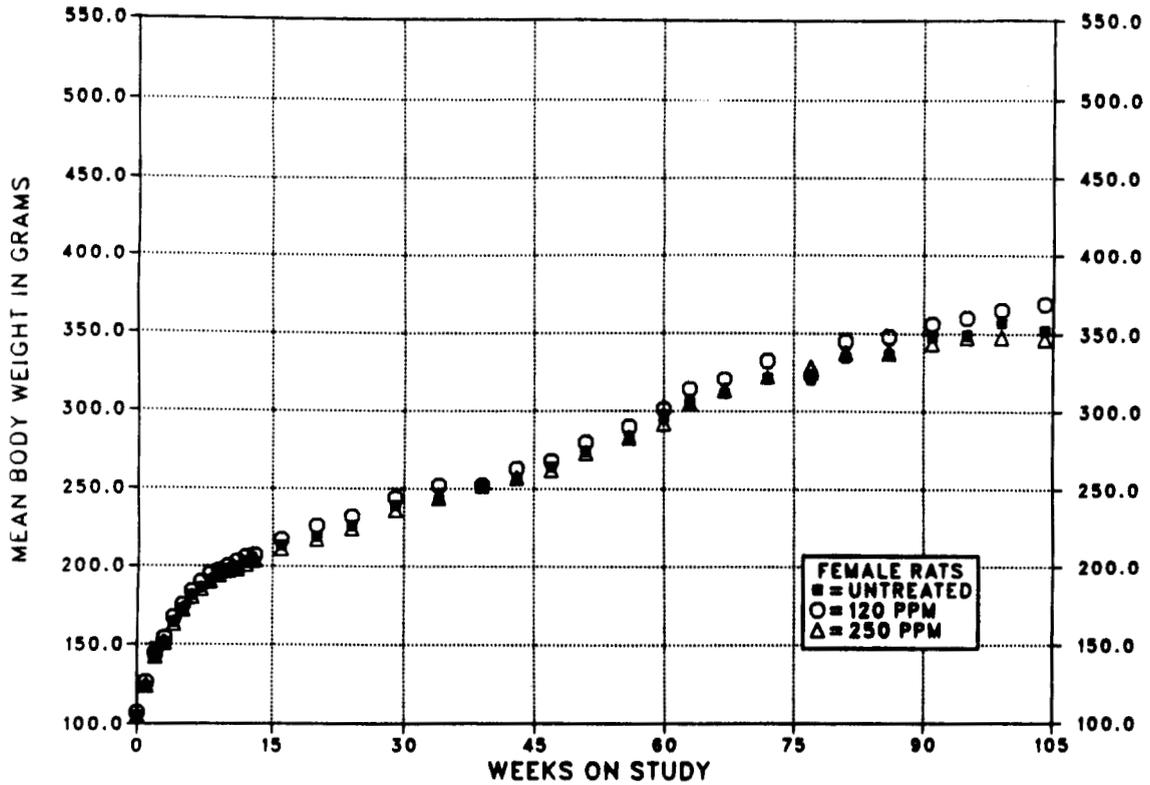
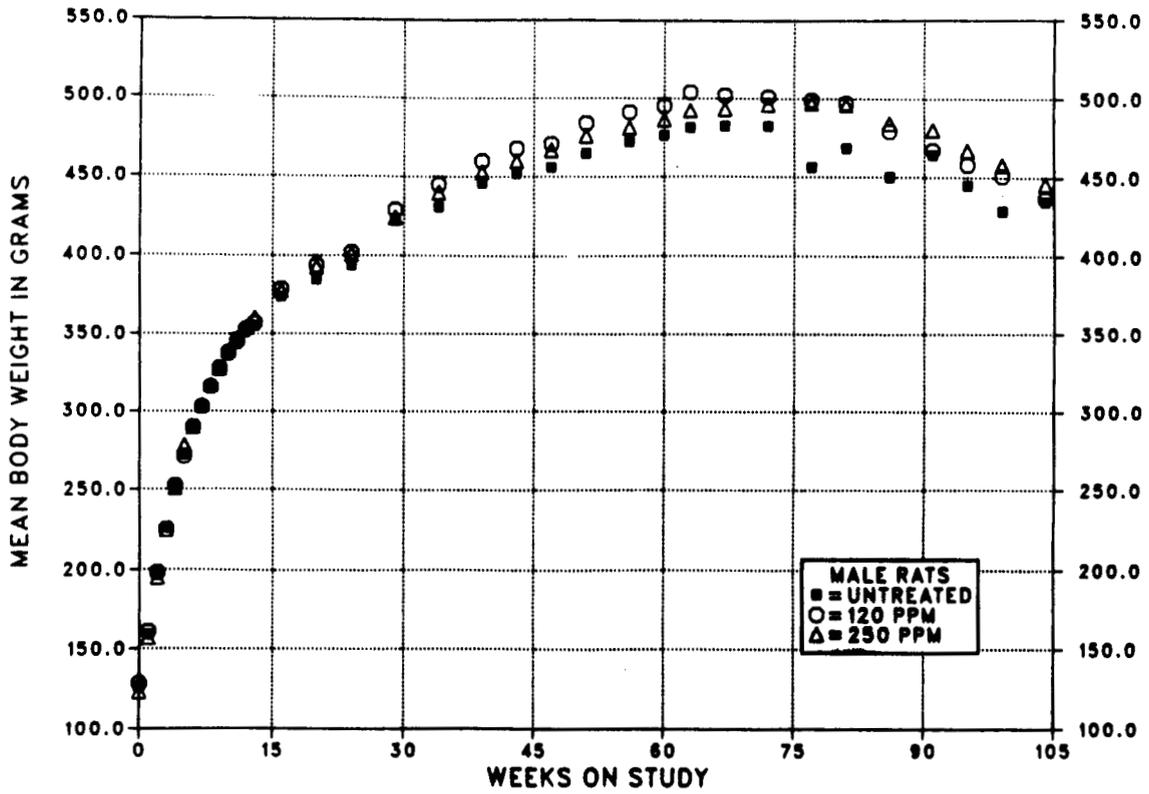


FIGURE 3. GROWTH CURVES FOR RATS FED DIETS CONTAINING RHODAMINE 6G FOR TWO YEARS

III. RESULTS: RATS

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.

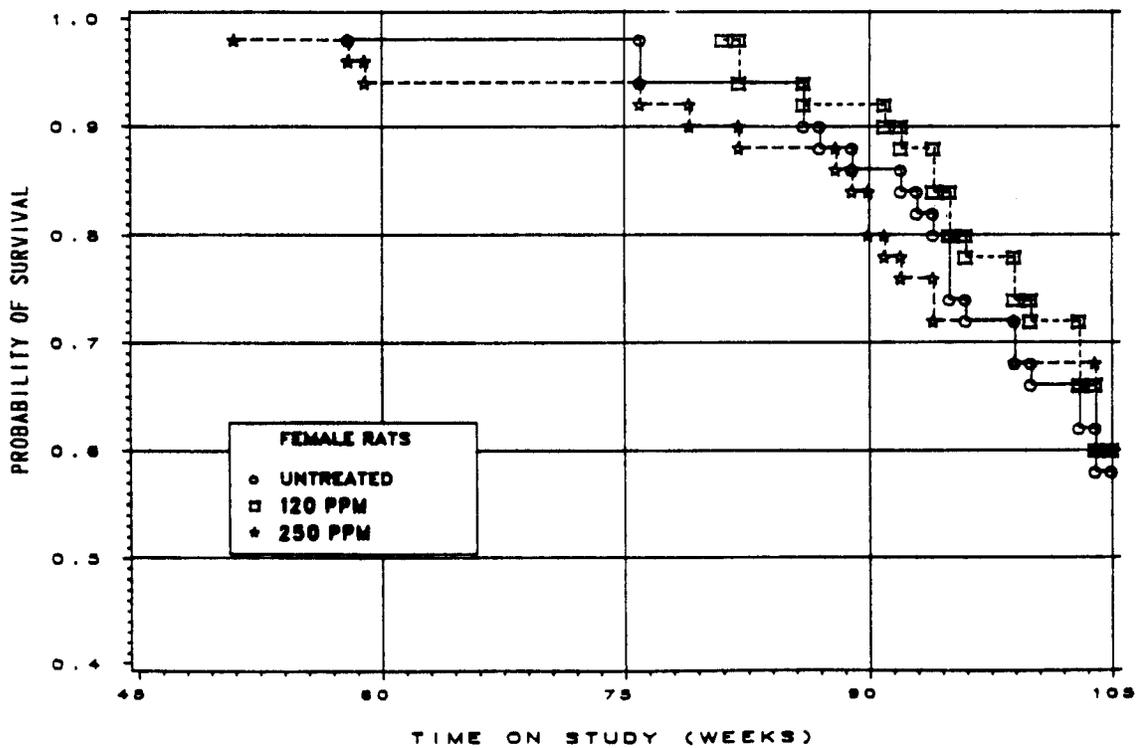
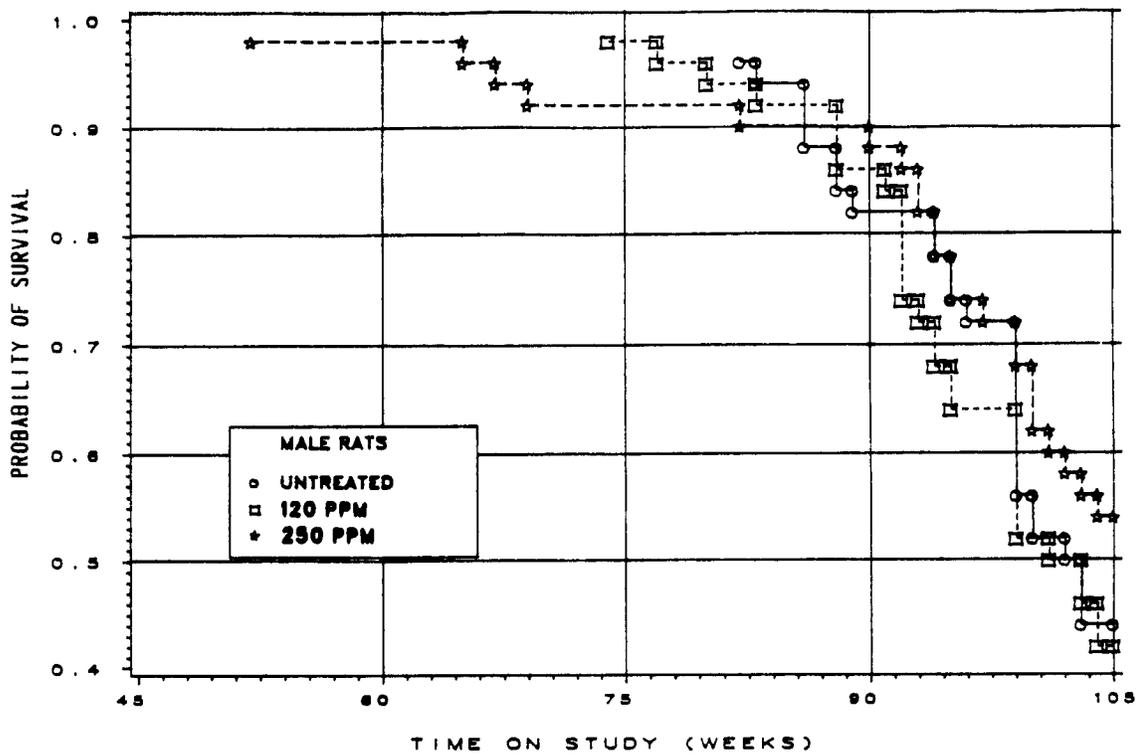


FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING RHODAMINE 6G FOR TWO YEARS

III. RESULTS: RATS

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 \pm SD): 12/439 (3% \pm 5%); historical incidence in NTP studies: 31/1,936 (2% \pm 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 \pm SD): 26/436 (6% \pm 5%); historical incidence in NTP studies: 99/1,968 (5% \pm 4%)

III. RESULTS: MICE

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 (Ta-

ble 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; LD₅₀ values by probit analysis.

(b) Initial group mean body weight ± standard error of the mean

(c) LD₅₀ = 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) LD₅₀ = 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) | | | Final Weight Relative to Controls (percent) | Feed Consumption (d) | |
|---------------------|--------------|---------------------------|------------|------------|---|----------------------|--------|
| | | Initial (b) | Final | Change (c) | | 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) | | | Final Weight Relative to Controls (percent) | Feed Consumption (d) | |
|---------------------|--------------|---------------------------|------------|------------|---|----------------------|---------|
| | | Initial (b) | Final | Change (c) | | 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 | | Low Dose | | | High Dose | | |
|----------------|-----------------|------------------|------------------|---------------------------|------------------|------------------|---------------------------|------------------|
| | 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 | | | | | | | | |
| | | | 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 | 96 | 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 |

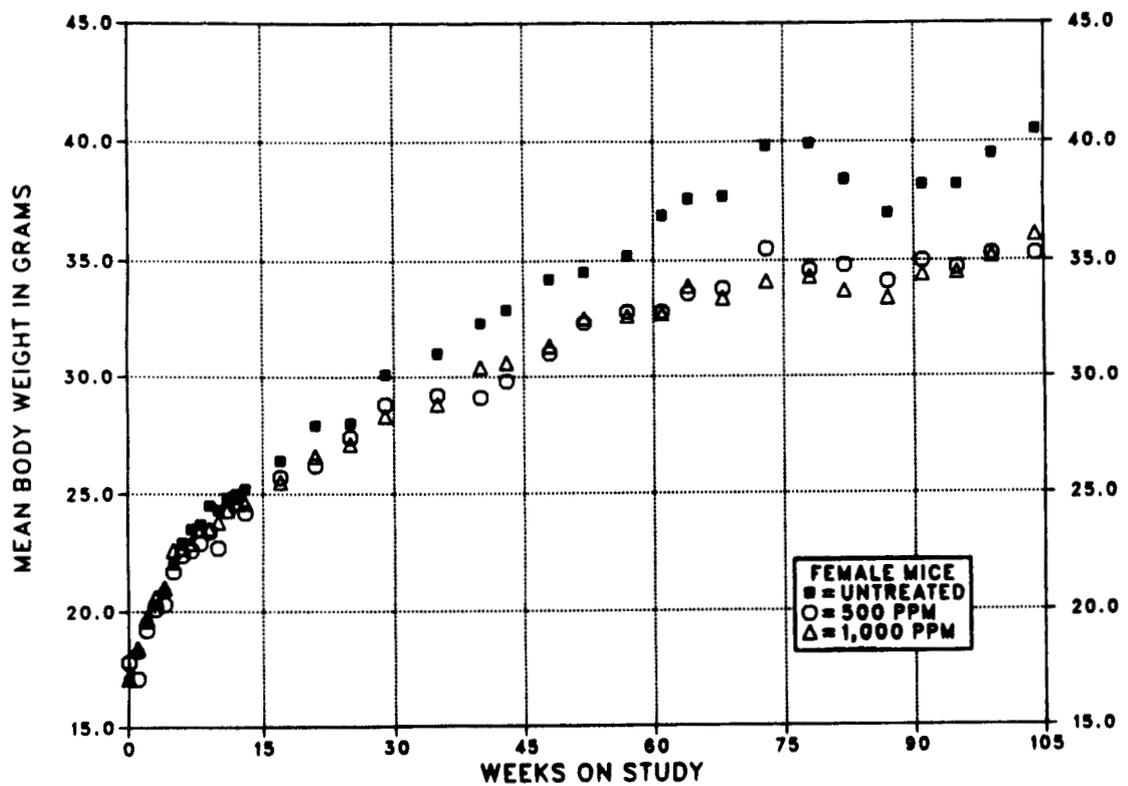
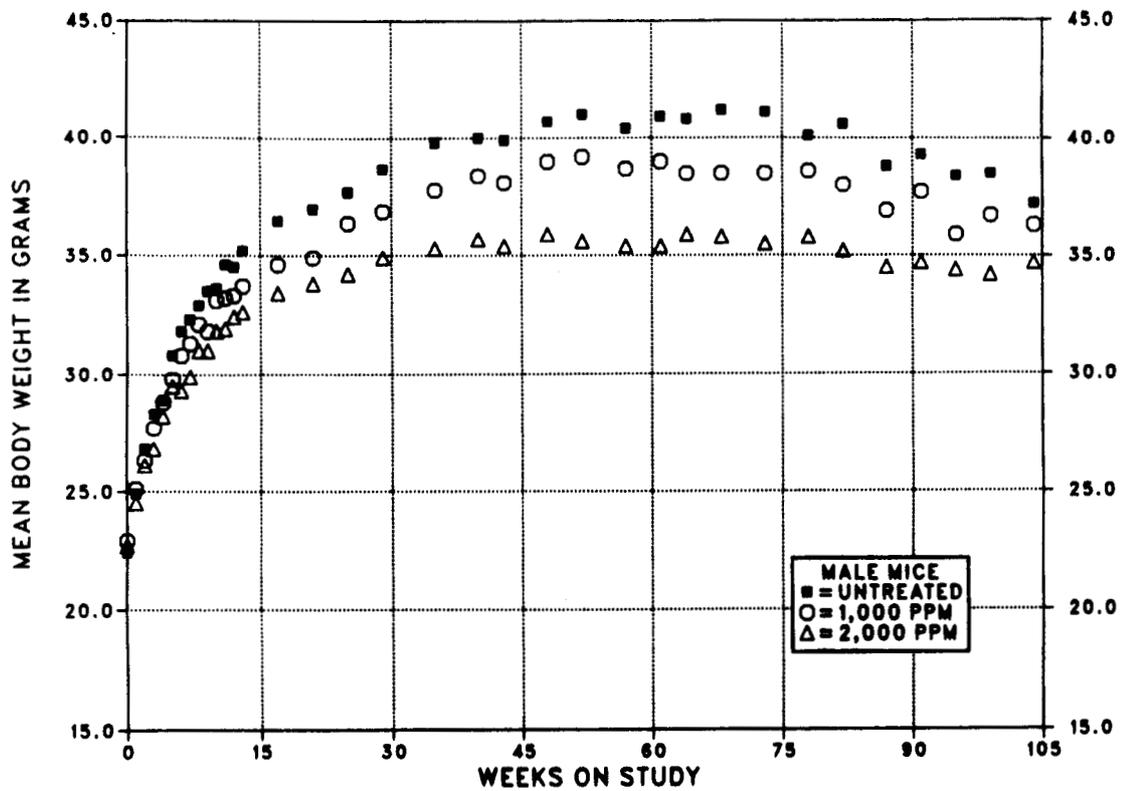


FIGURE 5. GROWTH CURVES FOR MICE FED DIETS CONTAINING RHODAMINE 6G FOR TWO YEARS

III. RESULTS: MICE

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.

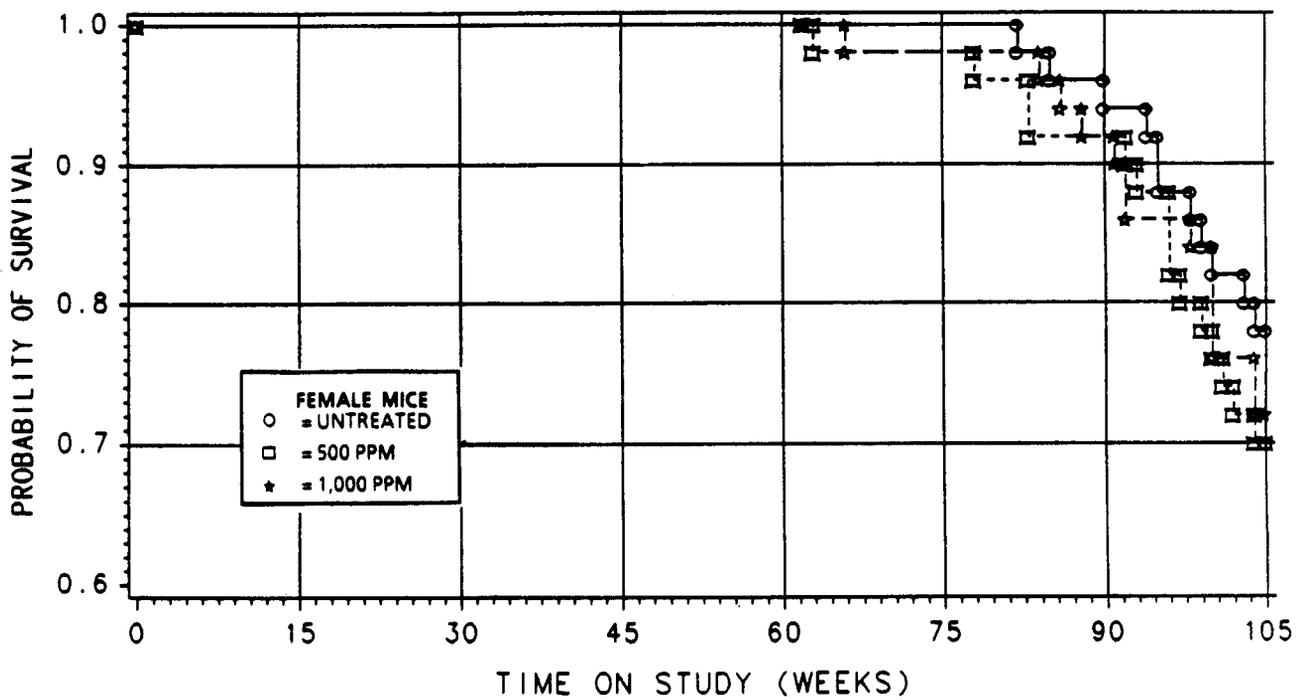
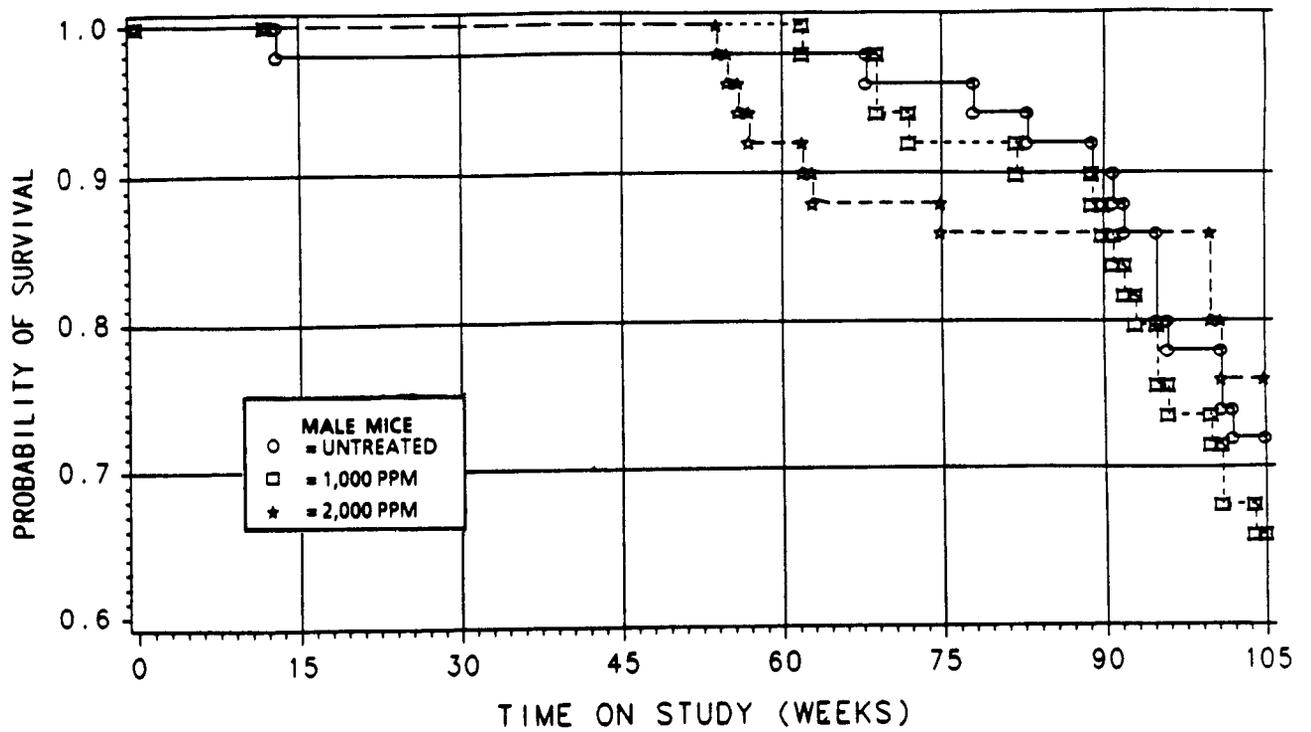


FIGURE 6. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING RHODAMINE 6G FOR TWO YEARS

III. RESULTS: MICE

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 inci-

dences 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 \pm SD): 11/434 (3% \pm 2%); historical incidence in NTP studies: 29/1,958 (1% \pm 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 \pm SD): 10/448 (2% \pm 2%); historical incidence in NTP studies: 48/2,040 (2% \pm 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 \pm SD): 104/448 (23% \pm 7%); historical incidence in NTP studies: 636/2,040 (31% \pm 13%)

III. RESULTS: GENETIC TOXICOLOGY

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 | | +S9 (hamster) | | +S9 (rat) | |
| | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | 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 |
| 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 |
| 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 |
| 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 \pm 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 TRIFLUOROTHYMININE 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 TRIFLUOROTHYMININE 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 \pm 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 | 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 | | | | | Trial 2--Harvest time: 20 hours (d) | | | | | | |
| Ethanol | 200 | 5 | 0.03 | 2.5 | Ethanol | 200 | 16 | 0.08 | 7.5 | | |
| Rhodamine 6G | | | | | Rhodamine 6G | | | | | | |
| 1.96 | 200 | 3 | 0.02 | 1.5 | 9.9 | 200 | 17 | 0.09 | 8 | | |
| 5.9 | 200 | 3 | 0.02 | 1.5 | 14.9 | 200 | 45 | 0.23 | 13.5 | | |
| 19.6 | 200 | 5 | 0.03 | 2.5 | 19.9 | 200 | 42 | 0.21 | 9.5 | | |
| 39.2 | 0 | | | | 29.9 | 0 | | | | | |
| Summary: Negative | | | | | Summary: Negative | | | | | | |
| Cyclophosphamide | 50 | 50 | 32 | 0.64 | 24 | Cyclophosphamide | 50.0 | 10 | 89 | 8.90 | 100 |
| Trial 3--Harvest time: 20.5 hours (d) | | | | | Trial 4--Harvest time: 20 hours (d) | | | | | | |
| Ethanol | 200 | 2 | 0.01 | 1 | Ethanol | 200 | 4 | 0.02 | 2 | | |
| Rhodamine 6G | | | | | Rhodamine 6G | | | | | | |
| 10 | 200 | 8 | 0.04 | 4 | 9.9 | 200 | 5 | 0.03 | 2.5 | | |
| 15 | 200 | 16 | 0.08 | 6 | 19.8 | 200 | 91 | 0.46 | 17 | | |
| 20 | 200 | 43 | 0.22 | 7.5 | 29.7 | 200 | 168 | 0.84 | 28 | | |
| 25 | 0 | | | | | | | | | | |
| Summary: Positive | | | | | Summary: Positive | | | | | | |
| Cyclophosphamide | 50 | 10 | 84 | 8.40 | 100 | 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 Matala 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 B6C3F₁ 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

IV. DISCUSSION AND CONCLUSIONS

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

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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 B6C3F₁ mice administered 1,000 or 2,000 ppm rhodamine 6G in the diet. There was *no evidence of carcinogenic activity* for female B6C3F₁ 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.

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APPENDIX A

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

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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 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8 8 8 8 8 8 8 8 8 8 8 8 8 8 9 9 9 9 9 9 9 9 9 9 | | | | | | | | | | | | | | | | | | | | | | |
| CARCASS ID | 2 3 4 6 7 7 9 9 9 5 5 6 6 6 0 0 0 0 0 0 0 0 0 0 | | | | | | | | | | | | | | | | | | | | | | |
| | 7 4 8 5 4 7 2 2 5 9 6 8 1 0 4 6 7 0 2 4 9 9 8 1 5 | | | | | | | | | | | | | | | | | | | | | | |
| 1 1 1 1 2 2 1 2 2 1 1 2 1 1 3 2 3 2 3 4 2 3 3 2 3 | | | | | | | | | | | | | | | | | | | | | | | |
| 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 |
| 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 | | + | + | + | + |
| Cortex, adenoma | | | | | | | X | | | | | | | | | | | | | | | | |
| Medulla, pheochromocytoma malignant | | | | | | | | | | | | | | | | | | X | | X | | | X |
| Medulla, pheochromocytoma malignant, multiple | | | | | | | | | | | | | | | | | | | X | | X | | X |
| Medulla, pheochromocytoma benign | | | | X | | | | X | | | | | | X | X | | | X | X | X | | X | X |
| Medulla, pheochromocytoma benign, multiple | | | | | | | | | | | | | X | | | | | | | | | | |
| Islets, pancreatic | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid gland | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pituitary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Leukemia mononuclear | | | X | | | | X | X | | | | | | | | | | | | | | | |
| Pars distalis, adenoma | X | X | | | | | | | | | | | | | | | | | | | | | |
| Thyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Leukemia mononuclear | | | | | | | X | | | | | | | | | | | | | | | | |
| C-cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | |
| C-cell, carcinoma | | | | | | | | | | | | | | | | | | | | | | | |
| Follicular cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | |
| Follicular cell, carcinoma | | | | | | | | | | | | | | | | | | | | | | | |
| GENERAL BODY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| None | | | | | | | | | | | | | | | | | | | | | | | |
| GENITAL SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Epididymis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | M | + | + | + | + | + |
| Preputial gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | M | + | + | + | + |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Schwannoma malignant | | | | | | | | | | | | | | | | | | | | | | | |
| Testes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Leukemia mononuclear | | | | | | | | | | | | | | | | | | | | | | | |
| Interstitial cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | |
| Interstitial cell, adenoma, multiple | | | | | | | | | | | | | | | | | | | | | | | |
| Tunic, mesothelioma malignant | X | | X | | X | X | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | 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 A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF RHODAMINE 6G: LOW DOSE

| WEEKS ON STUDY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | |
|--|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CARCASS ID | 7 | 7 | 8 | 8 | 8 | 8 | 8 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 0 | 0 | 0 | 0 | 0 | |
| | 5 | 7 | 0 | 4 | 9 | 9 | 9 | 2 | 2 | 3 | 3 | 3 | 3 | 4 | 5 | 5 | 5 | 6 | 9 | 9 | 0 | 0 | 0 | 0 | 2 | |
| ALIMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine large | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Serosa, mesothelioma malignant | | | X | | | | | | | | | | | | | | | | | | | | | | | |
| Intestine small | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Ileum, polyp adenomatous | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 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 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Leukemia mononuclear | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acinus, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acinus, adenoma, multiple | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Serosa, mesothelioma malignant | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary glands | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Leukemia mononuclear | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Papilloma squamous | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 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 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Medulla, leukemia mononuclear | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Medulla, pheochromocytoma malignant | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Medulla, pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Medulla, pheochromocytoma benign, multiple | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Islets, pancreatic | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adenoma, multiple | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid gland | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Pituitary gland | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Leukemia mononuclear | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pars distalis, adenoma | X | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thyroid gland | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| C-cell, adenoma | | | | X | | | | | | | | | | | | | | | | | | | | | | |
| C-cell, carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| GENERAL BODY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| None | | | | | | | | | | | | | | | | | | | | | | | | | | |
| GENITAL SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Epididymis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Preputial gland | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Seminal vesicle | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Serosa, mesothelioma malignant | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Testes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Capsule, mesothelioma malignant | X | X | | | | | | | | | | | | | | | | | | | | | | | | |
| Interstitial cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Interstitial cell, adenoma, multiple | X | X | X | X | X | X | X | | | | | | | | | | | | | | | | | | | |

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

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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 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%) | | 1 (2%) |
| Fungus | | 2 (4%) | |
| 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

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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 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 B6C3F₁ 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

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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 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 B6C3F₁ 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 |
| o-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 B6C3F₁ 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 |
| o-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 | | | 1 (2%) |
| Mediastinal, inflammation, suppurative | 2 (4%) | | 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

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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 B6C3F₁ 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:

| | <u>Hemagglutination Inhibition</u> | <u>Complement Fixation</u> | <u>ELISA</u> |
|------|--|---|--|
| 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

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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 | 282 |
| 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)

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| TABLE G4 | CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION | 188 |

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 |
| <i>d</i> - α -Tocopheryl acetate | 20,000 IU | |
| Choline | 560.0 g | Choline chloride |
| Folic acid | 2.2 g | |
| Niacin | 30.0 g | |
| <i>d</i> -Pantothenic acid | 18.0 g | <i>d</i> -Calcium pantothenate |
| Riboflavin | 3.4 g | |
| Thiamine | 10.0 g | Thiamine mononitrate |
| B ₁₂ | 4,000 μ g | |
| Pyridoxine | 1.7 g | Pyridoxine hydrochloride |
| Biotin | 140.0 mg | <i>d</i> -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 \pm Standard Deviation | Range | Number of Samples |
|--|-------------------------------|-----------------|-------------------|
| Crude protein (percent by weight) | 24.25 \pm 1.04 | 22.6-26.3 | 24 |
| Crude fat (percent by weight) | 5.10 \pm 0.44 | 4.4-6.0 | 24 |
| Crude fiber (percent by weight) | 3.38 \pm 0.38 | 2.4-4.2 | 24 |
| Ash (percent by weight) | 6.59 \pm 0.34 | 5.97-7.42 | 24 |
| Amino Acids (percent of total diet) | | | |
| Arginine | 1.323 \pm 0.830 | 1.21-1.39 | 4 |
| Cystine | 0.310 \pm 0.099 | 0.218-0.400 | 4 |
| Glycine | 1.155 \pm 0.069 | 1.06-1.21 | 4 |
| Histidine | 0.572 \pm 0.030 | 0.530-0.603 | 4 |
| Isoleucine | 0.910 \pm 0.033 | 0.881-0.944 | 4 |
| Leucine | 1.949 \pm 0.065 | 1.85-1.99 | 4 |
| Lysine | 1.275 \pm 0.076 | 1.20-1.37 | 4 |
| Methionine | 0.422 \pm 0.187 | 0.306-0.699 | 4 |
| Phenylalanine | 0.909 \pm 0.167 | 0.665-1.04 | 4 |
| Threonine | 0.844 \pm 0.029 | 0.824-0.886 | 4 |
| Tryptophan | 0.187 | 0.171-0.211 | 3 |
| Tyrosine | 0.631 \pm 0.094 | 0.566-0.769 | 4 |
| Valine | 1.11 \pm 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 \pm 1,239 | 8,900-1,400 | 24 |
| Vitamin D (IU/kg) | 4,650 | 3,000-6,300 | 2 |
| α -Tocopherol (ppm) | 41.53 \pm 7.52 | 31.1-48.9 | 4 |
| Thiamine (ppm) | 16.2 \pm 2.30 | 12.0-21.0 | (b) 23 |
| Riboflavin (ppm) | 7.5 \pm 0.96 | 6.1-8.2 | 4 |
| Niacin (ppm) | 85.0 \pm 14.2 | 65.0-97.0 | 4 |
| Pantothenic acid (ppm) | 29.3 \pm 4.6 | 23.0-34.0 | 4 |
| Pyridoxine (ppm) | 7.6 \pm 1.5 | 5.6-8.8 | 4 |
| Folic acid (ppm) | 2.8 \pm 0.88 | 1.8-3.7 | 4 |
| Biotin (ppm) | 0.27 \pm 0.05 | 0.21-0.32 | 4 |
| Vitamin B ₁₂ (ppb) | 21.0 \pm 11.9 | 11.0-38.0 | 4 |
| Choline (ppm) | 3,302.0 \pm 120.0 | 3,200.0-3,430.0 | 4 |
| Minerals | | | |
| Calcium (percent) | 1.23 \pm 0.12 | 1.10-1.53 | 24 |
| Phosphorus (percent) | 0.97 \pm 0.06 | 0.84-1.10 | 24 |
| Potassium (percent) | 0.862 \pm 0.100 | 0.772-0.974 | 3 |
| Chloride (percent) | 0.546 \pm 0.100 | 0.442-0.635 | 4 |
| Sodium (percent) | 0.311 \pm 0.038 | 0.258-0.350 | 4 |
| Magnesium (percent) | 0.169 \pm 0.133 | 0.151-0.181 | 4 |
| Sulfur (percent) | 0.316 \pm 0.070 | 0.270-0.420 | 4 |
| Iron (ppm) | 447.0 \pm 57.3 | 409.0-523.0 | 4 |
| Manganese (ppm) | 90.6 \pm 8.20 | 81.7-95.5 | 4 |
| Zinc (ppm) | 53.6 \pm 5.27 | 46.1-58.6 | 4 |
| Copper (ppm) | 10.77 \pm 3.19 | 8.09-15.39 | 4 |
| Iodine (ppm) | 2.95 \pm 1.05 | 1.52-3.82 | 4 |
| Chromium (ppm) | 1.81 \pm 0.28 | 1.44-2.09 | 4 |
| Cobalt (ppm) | 0.68 \pm 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 |
| <i>E. coli</i> (MPN/g) (g) | 8.0 ± 7.91 | <3-23 | 23 |
| <i>E. coli</i> (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 |
| <i>N</i> -Nitrosodimethylamine (ppb) (i, l) | 3.82 ± 4.29 | 0.6-16.8 | 22 |
| <i>N</i> -Nitrosodimethylamine (ppb) (i, m) | 11.71 ± 27.03 | 0.6-99 | 24 |
| <i>N</i> -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.