NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 220

CARCINOGENESIS BIOASSAY OF C. I. ACID RED 14

(CAS NO. 3567-69-9)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDY)

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

NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of chemically induced disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/ validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is comprised of four charter DHHS agencies: the National Cancer Institute, National Institutes of Health; the National Institute of Environmental Health Sciences, National Institutes of Health; the National Center for Toxicological Research, Food and Drug Administration; and the National Institute for Occupational Safety and Health, Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS.

NTP Technical Report

on the

CARCINOGENESIS BIOASSAY

of

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IN F344 RATS AND B6C3F$_1$ MICE

(FEED STUDY)

NATIONAL TOXICOLOGY PROGRAM
Research Triangle Park
P.O. Box 12233
North Carolina 27709
and
Bethesda, Maryland 20205

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NOTE TO THE READER

This is one in a series of experiments designed to determine whether selected chemicals produce cancer in animals. Chemicals selected for testing in the NTP carcinogenesis bioassay program are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

This study was initiated by the National Cancer Institute's Carcinogenesis Testing Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program.

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Comments and questions about the National Toxicology Program Technical Reports on Carcinogenesis Bioassays should be directed to the National Toxicology Program, located at Room A-306, Landow Building, Bethesda, MD 20205 (301-496-1152) or at Research Triangle Park, NC 27709 (919-541-3991).

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to communicate any mistakes to the Deputy Director, NTP (P.O. Box 12233, Research Triangle Park, NC 27709), so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP.

TABLE OF CONTENTS

TABLES

FIGURES

APPENDIXES

ABSTRACT

A carinogenesis bioassay of textile grade C.I. Acid Red 14 (67%-71% purity) was conducted by feeding diets containing 6,000 or 12,500 ppm of this dye for 103-104 weeks to groups of 50 male F344 rats, 12,500 or 25,000 ppm to groups of 50 female F344 rats, and 3,000 or 6,000 ppm to groups of 50 B6C3F1 mice of either sex. Groups of 90 untreated rats of either sex and 50 untreated mice of either sex served as controls.

Throughout the study, mean body weights of dosed rats of either sex and of dosed female mice were comparable with those of the controls, while the mean body weight of high-dose male mice was slightly lower than that of the controls.

Fourteen male rats in the low-dose group and 2 in the high-dose group accidentally drowned between weeks 84 and 103; 56% and 60% of these groups survived to terminal kill compared with 78% of the controls. These losses may have reduced the sensitivity of the assay in male rats.

Rats and mice may have tolerated higher doses, but the slight depression of mean body weight in high-dose male mice and the nonneoplastic lesions observed in dosed female mice and in rats of both sexes suggest that doses administered in this study could be considered maximum tolerated doses.

Endometrial stromal polyps of the uterus were observed in high-dose female rats at an incidence significantly higher (P=0.008) than that seen in the controls (controls: 9/87, 10%; low-dose: 11/50, 22%; high-dose: 14/50, 28%). However, the observed incidence of this tumor in the dosed groups was similar to the historical rate in untreated female F344 rats at this laboratory (65/286, 23%; range 10%-37%). Hence, the increased incidence of this lesion is not regarded as being associated with the administration of C.I. Acid Red 14.

Administration of C.I. Acid Red 14 to mice was not associated with an increased incidence of any tumor type.

Under the conditions of this bioassay, C.I. Acid Red 14 was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

CONTRIBUTORS

This carcinogenesis bioassay of C.I. Acid Red 14 was conducted from December 1976 to January 1979 at Battelle Columbus Laboratories, Columbus, Ohio, under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NTP Carcinogenesis Testing Program.

Dr. A. Peters (1) was the principal investigator for this study. Doses of the test chemical were selected by Drs. A. Peters and J. Robens (2,3). Drs. A. Peters, H. Harroff (1), and P. Stromberg (1) were in charge of animal care.

Necropsies were directed by Drs. G. Dill (1), R. Persing (1), R. Everett (1,4), and D. Thake (1). Histopathologic evaluations were performed by Drs. G. Dill (rats) and R. Persing (mice) (1). The pathology report and selected slides were evaluated by the NTP Pathology Working Group as described by Ward et al. (1978). The diagnoses represent a consensus of contracting pathologists and the NTP Pathology Working Group with final approval by the NTP Pathology Working Group.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (5). Statistical analyses were performed by Dr. J. R. Joiner (2) and Mr. J. Warner (2) using methods selected for the bioassay program by Dr. J. J. Gart (6). Original analyses of the test chemical were conducted at Midwest Research Institute (7). Bulk chemical reanalysis and dosage analysis were conducted at Battelle Columbus Laboratories and supervised by Drs. R. Freudenthal (1) and P. Leber (1,8) and Mr. D. Emmerling (1).

This report was prepared at Tracor Jitco (2) under the direction of Dr. C. Cueto (9), Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. M. A. Stedham, pathologist; Dr. A. C. Jacobs, bioscience writer; and Dr. W. D. Theriault and Ms. M. W. Glasser, technical editors.

The following scientists at NCI/NTP (10) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Michael P. Dieter, Dr. J. Fielding Douglas, Dr. Charles Grieshaber, Dr. Larry Hart, Dr. Joseph Haseman, Dr. James Huff, Dr. Richard Irwin (Chemical Manager), Dr. C. W. Jameson, Dr. Mary R. Kornreich, Dr. Eugene E. McConnell, Dr. John Mennear, Dr. John A. Moore, Dr. Raymond Tennant, and Dr. Jerrold M. Ward.

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PEER REVIEW COMMENTS

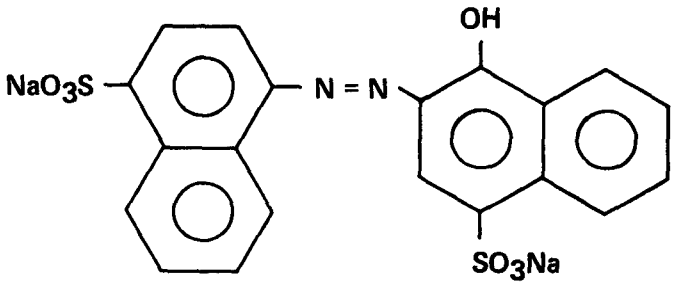
On October 15, 1980 this report underwent peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting began at 9 a.m. in Room 6, Building 31C, National Institutes of Health, Bethesda, Maryland. Members of the Subcommittee are: Drs. Margaret Hitchcock (Chairperson), Curtis Harper, Thomas Shepard, and Alice Whittemore. Members of the Panel are: Drs. Norman Breslow, Joseph Highland, Charles Irving, Frank Mirer, Sheldon Murphy, Svend Nielsen, Bernard Schwetz, Roy Shore, James Swenberg, and Gary Williams. Drs. Highland, Irving, Whittemore, and Williams were unable to attend the review.

Dr. Breslow, a principal reviewer for the report on the carcinogenesis bioassay of C.I. Acid Red 14, agreed with the conclusion in the report that C.I. Acid Red 14 was not clearly shown to be carcinogenic for either F344 rats or B6C3F1 mice in this bioassay. He noted that the deaths by drowning of 14 male rats in the low-dose group and two in the high-dose group between weeks 84 and 103 were not taken into account in the statistical analysis. However, he felt that this shortcoming was unlikely to have altered the conclusions. [This report now includes the results of life table analyses, which adjust for premature deaths. These analyses did not produce any additional evidence of carcinogenicity.] Dr. Breslow also observed that the dose levels, especially for mice, were probably set too low.

Dr. Schwetz, a second principal reviewer, agreed with the conclusion that C.I. Acid Red 14 was not carcinogenic for either species. He emphasized that two separate batches of the compound, one of 67% purity and the other of 71% purity, were used in this bioassay. Further, the concentrations selected were at the MTD for male mice but not for female mice or rats of either sex. Thus, he thought the conclusions were appropriate for the data collected, but the study was not a strong challenge of the carcinogenicity of this dye.

Dr. Breslow moved that the report on the bioassay of C.I. Acid Red 14 be accepted after inclusion of certain identified modifications. Dr. Schwetz seconded the motion, and the report was approved unanimously by the Peer Review Panel.

I. INTRODUCTION



C.I. ACID RED 14 (CARMOISINE)

Molecular Weight: 502.5

Chemical Formula: $C_{20}H_{12}N_2Na_2O_7S_2$

Chemical Name: 4-hydroxy-3- (4-Sulfo-1-naphthalenyl)azo -1-naphthalemesulfonic acid, disodium

C.I. Acid Red 14 (CAS No. 3567-69-9) is a water soluble monoazo dye used on nylon, silk, wool, leather, acetate, cellulose, anodized aluminum, paper, and wood; some foods processed (before 1971) in Europe also contained C.I. Acid Red 14 (Society of Dyers and Colourists, 1971). First synthesized by Witt in 1883, large scale U.S. production of carmoisine was first reported in 1914; in 1921, ten U.S. manufacturers reported a total of 106,000 kg (IARC, 1975). In the United States, the dye was used in cosmetics and externally applied drugs until approval was withdrawn in October 1966 (CFR, 1974). At present, the composition of C.I. Acid Red 14 manufactured in the United States may vary according to the customer's shade and intensity requirements; thus, it is not produced according to rigid chemical specifications and may contain numerous inorganic salts. The

British Standards Institution (1960) and the FAO/WHO (1966) require a minimum of 85% carmoisine in the commercial product. Production of C.I. Acid Red 14 in the United States was 51,000 pounds in 1978 (USITC, 1979).

Intraperitoneal LD_{50} values of 1.1 g/kg for male E strain rats and 0.9 g/kg for male and female Alderly Park ICI mice have been reported, but single oral doses as high as 10 g/kg and 8 g/kg (in the same strains of rats and mice) have not produced death (Gaunt et al., 1967).

C.I. Acid Red 14 was not mutagenic in Salmonella typhimurium TA 1538, TA 1535, TA 1537, TA 100, and TA 98 with or without metabolic activation with S-9 microsomes (Garner and Nutman, 1977; Viola and Nosotti, 1978; and Brown et al., 1978).

Groups of 15 male and 15 female albino mice (strain not specified) received twice weekly subcutaneous injections of 0.1 ml of a 3% carmoisine suspension in arachis oil (6 mg/week) for 6 months; for the next 6 months, the concentration was doubled (6% suspension, 12 mg/week). The total dose at 52 weeks equaled 468 mg/mouse. Thereafter, mice were allowed to survive as long as possible. The control group (30 of each sex) was given 0.1 ml arachis oil. The authors concluded that "carmoisine induced no tumors" (Bonser et al., 1956).

IARC (1975) recorded three other studies (unpublished) in rats exposed via diet (0.2%), drinking water (1%), or subcutaneous injection (total dose 500 mg). In these 1957 studies, no tumors were reported.

Groups of 30 male and 30 female ASH/CS1 mice were fed diets containing 0.01%, 0.05%, 0.25%, or 1.25% carmoisine (purity > 85%) for 80 weeks. These concentrations are equivalent to 100, 500, 2,500, or 12,500 mg dye per kg diet. Groups of 60 males and 60 females were used as controls. Both weight gain, organ weights, and survival were comparable between control and test groups. Significantly decreased ($P < 0.001$) hemoglobin values were observed for the 0.25% and 1.25% males and for the 1.25% females. Under

these experimental conditions, carmoisine was not carcinogenic for ASH/CS1 mice (Mason et al., 1974; IARC, 1975).

In an experiment with exposure beginning in utero and extending for one year after birth, test groups of 30 males and 30 females received diets containing 0.35%, 0.8%, or 2.0% carmoisine (3,500, 8,000, or 20,000 mg dye per kg diet). Control groups contained 50 males and 50 females. Other than an increase in thyroid weights for males and females fed the 2.0% diet and a higher incidence of minimal bronchitis, tracheal inflammation, and lymph node congestion in males at the 2.0% level, no compound-related effects were observed (Holmes et al., 1978).

C.I. Acid Red 14 was selected for testing because of its widespread use and because previous studies for carcinogenicity of this compound were considered not definitve.

II. MATERIALS AND METHODS

A. Chemical

Textile grade C.I. Acid Red 14 (CAS No. 3567-69-9) -- 4-hydroxy-3-((4-sulfono-1-naphthalenyl)azo-1-naphthalenesulfonic acid, disodium salt -- was obtained from GAF (Linden, NJ) in two batches. Lot No. A77716 was used for the subchronic studies and the first 11 months of the chronic studies, and Lot No. A83650 was used for the final 13 months of the chronic studies.

Results of purity and identity analyses performed at Midwest Research Institute indicated that Lot No. A77716 was 71.4% dye (based on titration of the azo function), 7.4% water (based on a Karl Fisher analysis), and 11.7% sodium chloride (based on elemental analysis), although the values for sodium and sulfur were slightly higher than those calculated (Appendix E). Four trace or slight impurities were indicated by thin-layer chromatography and three impurities by high-pressure liquid chromatography. Lot No. A83650 was found to contain 67.3% dye, 7.48% water, 7.85% sodium chloride, and 12.21% sodium sulfate (calculated from elemental analysis); values for carbon and sodium were slightly higher than those calculated (Appendix F). A slight impurity was detected by thin-layer chromatography in two solvent systems, and a trace impurity with an area of 0.6% of the major peak was detected by high-pressure liquid chromatography. These impurities were not identified.

The infrared, ultraviolet, and nuclear magnetic resonance spectra were consistent with the structure. Since the percentages of the individual constituents of each batch do not add up to 100%, a theoretical composition was calculated for each batch (Appendix G). This calculation was based on the actual elemental analyses, the titration of the azo function, the Karl Fisher water analysis, and information about the manufacturing process.

The textile grade material, referred to in this report as C.I. Acid Red 14, was stored at 23°±1°C throughout the study.

B. Dietary Preparation

Feed samples containing 100,000 ppm C.I. Acid Red 14 were stable for 2 weeks at temperatures up to 45°C (Appendix I).

Test diets were formulated by mixing Purina® Laboratory Chow and the weighed amount of textile grade C.I. Acid Red 14 for 15 minutes in a Patterson-Kelly® twin-shell blender equipped with an intensifier bar. One-week supplies of each diet were prepared no more than 4 days before use and were stored at 23°C for no longer than 10 days. Control diets consisted of Purina® Laboratory Chow.

The concentration of C.I. Acid Red 14 in randomly selected batches of formulated diets was measured every 8 weeks and was found to be within ±10% of the desired concentration (Appendix H).

C. Animals

Fischer 344 rats and B6C3F1 mice were used in both the prechronic and the chronic studies. Four-week-old male rats, 3-week-old female rats, and 5-week-old mice were obtained from NCI Frederick Cancer Research Center, Frederick, Maryland, acclimated for 2 weeks, assigned randomly to cages, and these cages were randomly assigned to the various groups.

D. Animal Maintenance

Rats and mice were housed five per cage in solid-bottom polycarbonate cages (Table 1) supplied with DuPont 2024 spun bonded polyester filters and

Table 1. Specifications and Sources of Materials Used for Animal Maintenance

Item	Specifications	Source
Bedding	Absorb-dri® hardwood chips	Lab Products, Inc. (Garfield, NJ)
Cages	Solid bottom, polycarbonate	Lab Products, Inc. (Garfield, NJ)
Feed	Purina® meal Laboratory Chow	Ralston Purina Co. (Richmond, IN)
Watering System	Edstrom Automatic	Edstrom Industries (Waterford, WI)

hardwood chip bedding. Cages and bedding were changed twice weekly and feed hoppers once weekly. Rat cages were changed three times per week for the final 15 months of the chronic study. Water was supplied by an automatic watering system, and the test diet for the dosed animals and feed for the controls were available ad libitum. Temperature in the animal rooms was 22 to 24°C and the relative humidity was 45%-55%. Incoming air was passed through a filter equipped with an electrostatic precipitator. Animal rooms received at least 15 changes of air per hour. Fluorescent lighting was provided 12 hours per day.

Rats and mice fed C.I. Acid Red 14 were housed in the same room as animals of the same species on feeding studies of C.I. Acid Orange No. 10 (CAS 1936-15-8) and F D & C Yellow No. 6 (CAS 2783-94-0).

E. Single-Day Dosing and 14-Day Repeated Dose Studies

Single-day dosing and 14-day repeated-dose feed studies were conducted using F344 rats and B6C3F1 mice to determine the concentrations of C.I. Acid Red 14 to be used in the 90-day prechronic study.

In the single-day dosing study, groups of five males and five females of each species were fed diets containing 6,000, 12,500, 50,000, 100,000 or 200,000 ppm C.I. Acid Red 14 for 1 day and then lab chow for 13 days. All animals survived to the end of the 2-week test period. In the repeated-dose study, similar groups of rats and mice fed diets containing 6,000-100,000 ppm of the test chemical for 14 days also survived to the end of the test period (Tables 2 and 3); weight loss in male and female rats fed 100,000 ppm was the only compound-related effect observed.

F. 13-Week Study

Subchronic studies were conducted using F344 rats and B6C3F1 mice to determine the target organs and the concentrations to be used in the chronic

Table 2. Dosage, Survival, and Mean Body Weights of Rats Fed Diets Containing C.I. Acid Red 14 for 14 Days

	Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain
Male					
	6,000	5/5	137	138	1
	12,500	5/5	134	160	26
	25,000	5/5	142	153	11
	50,000	5/5	139	146	7
	100,000	5/5	142	134	-8
Female					
	6,000	5/5	112	118	6
	12,500	5/5	108	116	8
	25,000	5/5	110	116	6
	50,000	5/5	112	114	2
	100,000	5/5	111	98.2	-12.8

(a) Number surviving/number per group

Table 3. Dosage, Survival, and Mean Body Weights of Mice Fed Diets Containing C.I. Acid Red 14 for 14 Days

	Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain
Male					
	6,000	5/5	23.0	26.8	3.8
	12,500	5/5	21.8	25.6	3.8
	25,000	5/5	22.8	28.0	5.2
	50,000	5/5	22.4	25.2	2.8
	100,000	5/5	22.0	23.6	1.6
Female					
	6,000	5/5	19.0	20.0	1.0
	12,500	5/5	19.0	20.4	1.4
	25,000	5/5	18.2	20.2	2.0
	50,000	5/5	19.2	19.6	0.4
	100,000	5/5	19.4	19.2	-0.2

(a) Number surviving/number per group

studies. Diets containing 0, 6,000, 12,500, 25,000, 50,000, or 100,000 ppm ~~C.I. Acid Red~~ 14 were fed for 13 weeks to groups of five males or five females of each species (Tables 4 and 5). Clinical observations were made twice daily and individual animals were weighed weekly. At the end of the 91-day study, survivors were killed, necropsies were performed on all animals, and tissues (see Section H) were taken for histopathologic analysis.

Rats: Two male rats died, one that received 12,500 ppm and one that received 100,000 ppm. No deaths occurred among the females. Mean weight gain in male rats was depressed 11.3% in the 12,500-ppm group, 9.2% in the 25,000-ppm group, and 23% and 28% in the 50,000 and 100,000-ppm groups, and less than 10% in all female rats. Toxic nephrosis of renal proximal convoluted tubules, considered to be compound related, was observed in male rats fed 25,000, 50,000, or 100,000 ppm and in female rats fed 100,000 ppm. Based on the histopathologic effects observed in the kidney during the subchronic study, doses selected for rats in the chronic studies were 6,000 and 12,500 ppm C.I. Acid Red 14 for males and 12,500 and 25,000 ppm for females.

Mice: Two male mice (one receiving 25,000 ppm and one receiving 50,000 ppm) and one female control mouse died. Mean weight gain was depressed 78% among male mice fed 100,000 ppm, 53% among females fed 12,500 ppm, 24% among females fed 50,000 ppm, and 76% among females fed 100,000 ppm.

Spleens of male mice fed 100,000 ppm were enlarged up to 1.5 times their normal size. A dose-related increase in extramedullary hematopoiesis and hemosiderosis was observed in mice of either sex.

A generalized, but not strictly dose-related, increase in lymphocytic cystitis and an active-chronic cystitis were seen in urinary bladders of mice of either sex (Table 6). Neither type of cystitis was observed in control mice.

Based on the findings of urinary bladder lesions in the subchronic studies, doses selected for the mice in the chronic studies were 3,000 and 6,000 ppm.

Table 4. Dosage, Survival, and Mean Body Weights of Rats Fed Diets Containing C.I. Acid Red 14 for 13 Weeks

Dose (ppm)	Survival(a)	Mean Body Weights (grams)			Weight Change Relative to Controls (%) (b)
		Initial	Final	Gain	
Male					
0	10/10	95.2	290.6	195.4	
6,000	10/10	97.0	298.8	201.8	+3.2
12,500	9/10	98.6	272.0	173.4	-11.3
25,000	10/10	102.4	279.8	177.4	- 9.2
50,000	10/10	98.4	248.7	150.3	-23.1
100,000	9/10	87.3	228.1	140.8	-27.9
Female					
0	10/10	104.7	186.7	82.0	
6,000	10/10	99.7	182.7	83.0	+1.2
12,500	10/10	100.0	178.4	78.4	-4.4
25,000	10/10	96.2	175.5	79.3	-3.3
50,000	10/10	90.9	174.0	83.1	+1.3
100,000	10/10	88.7	164.0	75.3	-8.2

(a) Number surviving/number group

(b) Weight change relative to controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

Table 5. Dosage, Survival, and Mean Body Weights of Mice Fed Diets Containing C.I. Acid Red 14 for 13 Weeks

Dose (ppm)	Survival(a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (%) (b)
Male					
0	10/10	18.5	29.4	10.9	
6,000	10/10	18.6	30.0	11.4	+4.6
12,500	10/10	18.1	29.7	11.6	+6.4
25,000	9/10	18.5	29.6	11.1	+1.8
50,000	9/10	19.0	29.0	10.0	-8.3
100,000	10/10	18.6	21.0	2.4	-78.0
Female					
0	9/10	15.9	22.9	7.0	
6,000	10/10	16.0	23.6	7.6	+8.6
12,500	10/10	16.0	19.3	3.3	-52.9
25,000	10/10	16.4	22.9	6.5	-7.1
50,000	10/10	16.7	22.0	5.3	-24.3
100,000	10/10	16.7	18.4	1.7	-75.7

(a) Number surviving/number group

(b) Weight change relative to controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

Table 6. Incidence of Lesions in the Urinary Bladders of Mice Fed Diets Containing C.I. Acid Red 14 for 13 Weeks

Dose (mg/kg)	Sex	Active-Chronic Cystitis (a)	Lymphocytic Cystitis (a)
0	M	0/8	0/8
	F	0/7	0/7
6000	M	0/10	1/10
	F	0/9	0/9
12,500	M	0/10	3/10
	F	0/8	6/8
25,000	M	0/9	4/9
	F	0/9	7/9
50,000	M	1/10	8/10
	F	1/10	8/10
100,000	M	8/9	4/9
	F	5/10	5/10

(a) $\frac{\text{Number affected}}{\text{Number examined}}$

G. Chronic Studies

The test groups, doses administered, and durations of the chronic studies are shown in Table 7.

H. Clinical Examinations and Pathology

All animals were observed twice daily to discern sick or moribund animals. Clinical examination and palpation for masses were performed each month, and the animals were weighed at least monthly. Moribund animals and animals that survived to the end of the bioassay were killed with carbon dioxide and necropsied.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin (abdominal), lungs and bronchi, trachea, bone, bone marrow (femur) and thigh muscle, spleen, lymph nodes, thymus, heart, salivary glands, liver, pancreas, esophagus, stomach, gall bladder (mice), duodenum, jejunum, ileum, cecum, colon, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain, epididymus, eye, and all tissue masses.

Necropsies were performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

Table 7. Experimental Design of Chronic Feeding Studies with C.I. Acid Red 14 in Rats and Mice

Test Group	Initial No. of Animals	Dose (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male Rats				
Control	90 (a)	0	0	104
Low-Dose	50	6,000	103	1
High-Dose	50	12,500	103	1
Female Rats				
Control	90 (a)	0	0	104
Low-Dose	50	12,500	103	2
High-Dose	50	25,000	103	2
Male Mice				
Control	50	0	0	103
Low-Dose	50	3,000	103	1
High-Dose	50	6,000	103	1
Female Mice				
Control	50	0	0	104
Low-Dose	50	3,000	103	1
High-Dose	49	6,000	103	1

(a) Controls were shared with feeding studies of C.I. Acid Orange 10 and FD and C Yellow No. 6 which were conducted concurrently.

I. Data Recording and Statistical Analyses

Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically 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) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is reported only when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) before histologic sampling or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When the results from two dosed groups are compared simultaneously with that for a control group, a correction to

ensure an overall significance level of 0.05 may be made. The Bonferroni inequality criterion (Miller, 1966) requires that the P values for any comparison be less than or equal to 0.025. When this correction was used, it is discussed in the narrative section. It is not presented in the tables, where the Fischer exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed. Life table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was killed was entered as the time point of tumor observation. The methods of Cox and of Tarone were used for the statistical tests of the groups. The statistical tests were one-tailed.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The lower and upper limits of the confidence interval of the relative risk have been included in the tables of

statistical analyses. The interpretation of the limits is that, in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result has occurred (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero). When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

Statistical analyses and interpretations of the Carcinogenesis Bioassay data, issues of false positivity and false negativity, and time-adjusted analyses of incidence rates are discussed in Gart et al. (1979).

III. RESULTS - RATS

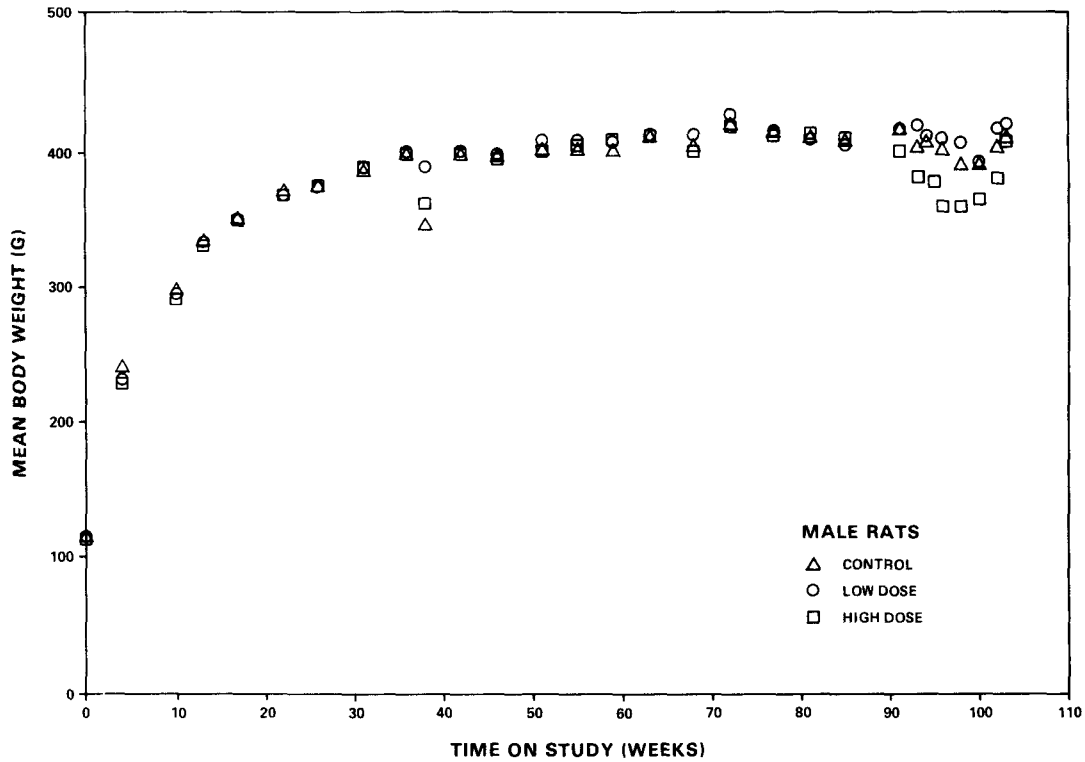
A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed rats of either sex were comparable with those of the controls throughout most of the study (Figure 1). No compound-related clinical signs were observed.

B. Survival (Rats)

Estimates of the probabilities of survival of male and female rats fed diets containing C.I. Acid Red 14 at the concentrations of this bioassay, together with those of the control group, are shown by the Kaplan and Meier curves in Figure 2. The survival of the low-dose group of male rats was significantly greater than that of the controls ($P=0.046$) or of the high-dose group ($P < 0.001$). No significant differences were observed between the control and high-dose groups of male rats or between any groups of female rats. Fourteen low-dose and 2 high-dose male rats drowned as a result of a failure in the automatic watering system during the study. Animals accidentally killed were censored from the estimates of the probability of survival (Figure 2), but were included in other statistical analyses.

In male rats, 70/90 (78%) of the controls, 28/50 (56%) of the low-dose, and 30/50 (60%) of the high-dose lived to the termination period of the study at 104 weeks. In female rats, 66/88 (75%) of the controls, 41/50 (82%) of the low-dose, and 38/50 (76%) of the high-dose group lived to the termination period of the study at 104 weeks. These incidences include animals accidentally killed.



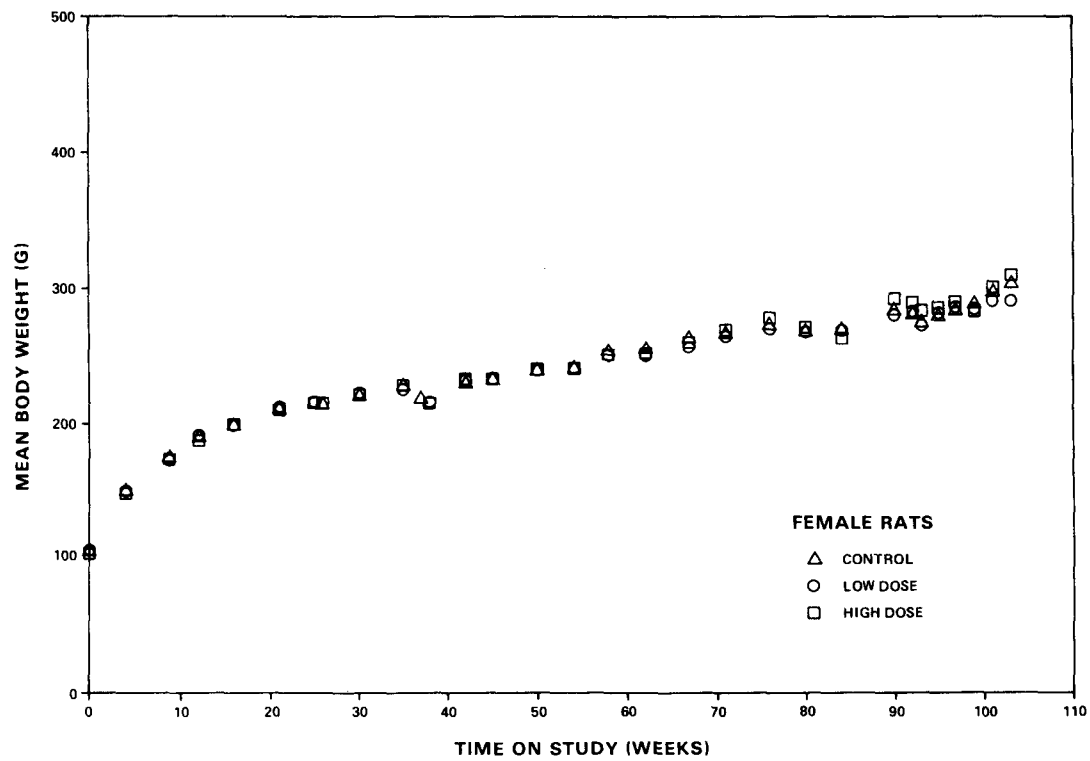


Figure 1. Growth Curves for Rats Fed Diets Containing C.I. Acid Red 14

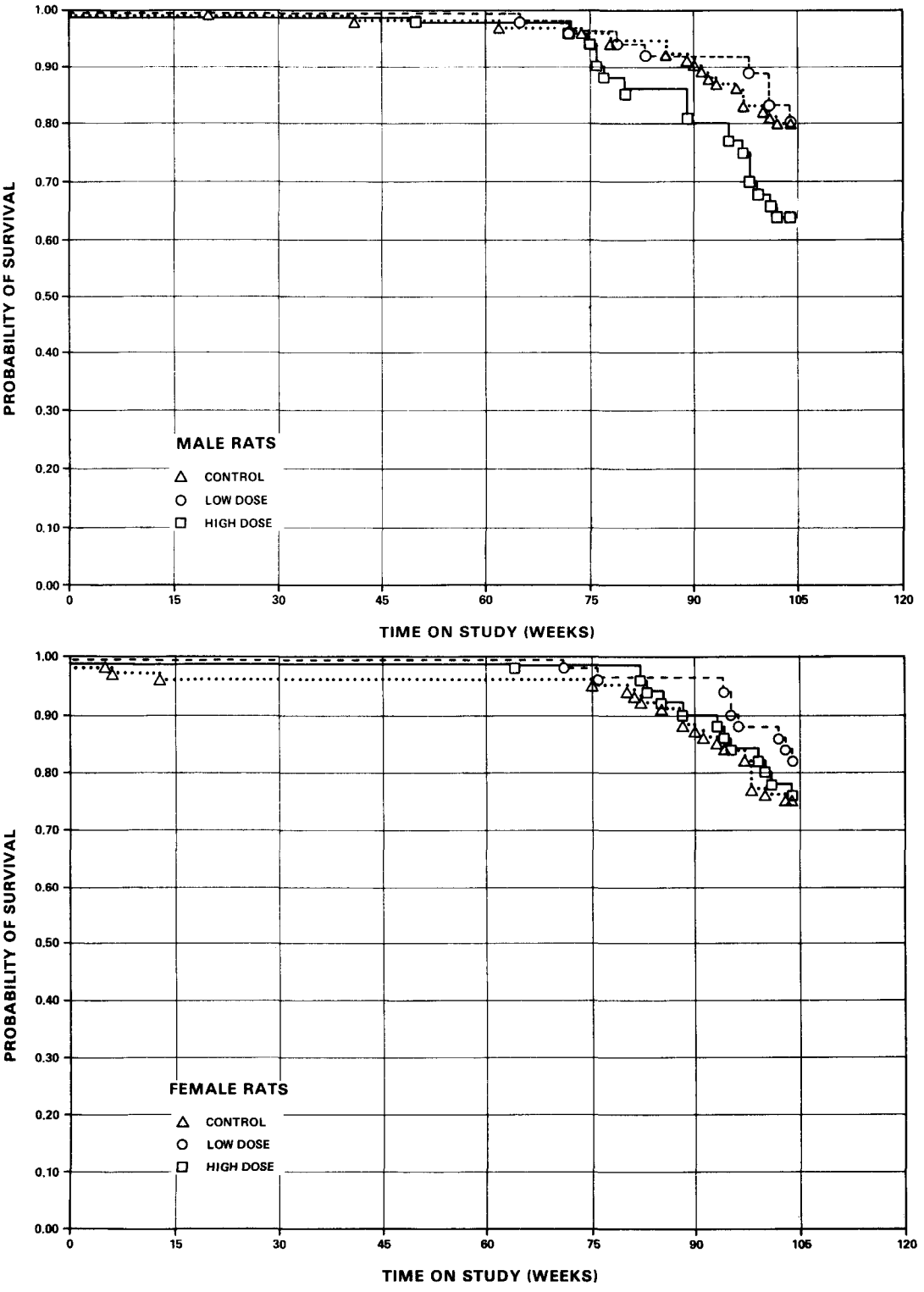


Figure 2. Survival Curves for Rats Fed Diets Containing C.I. Acid Red 14

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, Tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, Tables C1 and C2. Only tumors of the uterus appeared to be associated with administration of C.I. Acid Red 14. The incidence of endometrial stromal polyps of the uterus in dosed females was increased relative to controls (high dose: 14/50, 28%; low dose: 11/50, 22%; and controls: 9/87, 10%); however, the incidence in the high-dose group is within the range observed in control groups of aging F344 female rats in this laboratory (65/286, 23%).

The various nonneoplastic lesions represented among both control and dosed animals have been encountered previously as spontaneous occurrences in aging laboratory rats. An increased incidence of adrenal cortical focal hyperplasias, characterized by focal collections of basophilic, eosinophilic, or vacuolated cells, was seen in high-dose rats of both sexes (males: 5/89, 6%; 6/49, 12%; 8/50, 16%; females: 7/86, 8%; 7/50, 14%; 18/50, 36%).

Results of histopathologic examination indicated that C.I. Acid Red 14 was not carcinogenic to male or female F344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables 8 and 9 contain the statistical analyses of those primary tumors that occurred in at least two animals of one group and with an incidence of at least 5% in one or more groups.

Endometrial stromal polyps of the uterus in female rats were observed in a statistically significant positive relation (9/87, 10% in the controls; 11/50, 22% in the low-dose; and 14/50, 28% in the high-dose). The Cochran-Armitage test for linear trend was statistically significant in the positive direction (P=0.006). The Fisher exact test between the high-dose

group and the control group was significant (P=0.008); no statistically significant increase was observed in the low-dose group when compared to the control rate, but this tumor occurred more frequently in the low-dose group compared with the control group. The historical incidence of endometrial stromal polyps in untreated F344 female rats at this laboratory is 23% (65/286), with a range of 10%-37%. Because the incidence of this tumor in the dosed group is similar to the historical control rate, the observed increase in this lesion is not regarded as being associated with the administration of C.I. Acid Red 14.

Lymphomas or leukemia of the hematopoietic system in female rats were observed in decreased incidence in the low-dose group compared with the other two groups. The Cochran-Armitage test for linear trend was not significant. The Fisher exact test between the low-dose group and the control group was significant (P=0.043). The value of P=0.043 is above the value of P=0.025 required by the Bonferroni inequality criterion for an overall significance of P=0.05 when two dosed groups are compared with a common control group. No significant incidence is observed in the high-dose group. In male rats, this tumor was not observed in statistically significant proportions.

Chromophobe adenomas or carcinomas of the pituitary in female rats were observed in decreased incidence in the low-dose group compared with the other two groups. The Cochran-Armitage test for linear trend was not significant, but there was a departure from linear trend due to decreased incidence in the low-dose group compared with the other two groups. The Fisher exact test between the low-dose group and the control group was significant (P=0.037), but this value of P=0.037 is above the value of P=0.025 required by the Bonferroni inequality criterion for an overall significance of P=0.05 when two dosed groups are compared with a common control group. No significant incidence was observed in the high-dose group. In male rats, this tumor was not observed in a statistically significant proportion.

Time-adjusted tests, eliminating those animals dying before 52 weeks, and life table analyses did not produce any additional evidence of carcinogenicity.

Based on the statistical analysis, there was no site at which an increase in tumor incidence could be associated with the administration of the chemical.

Table 8. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing C.I. Acid Red 14 (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Lymphocytic Leukemia (b)	22/90(24)	7/50(14)	11/50(22)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.573	0.900
Lower Limit		0.220	0.427
Upper Limit		1.273	1.754
Weeks to First Observed Tumor	74	65	76
Hematopoietic System:			
Lymphomas or Leukemias (b)	23/90(26)	7/50(14)	11/50(22)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.548	0.861
Lower Limit		0.212	0.410
Upper Limit		1.208	1.664
Weeks to First Observed Tumor	20	65	76
Liver: Neoplastic			
Nodule (b)	5/90(6)	1/50(2)	2/49(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.360	0.735
Lower Limit		0.008	0.072
Upper Limit		3.079	4.276
Weeks to First Observed Tumor	104	104	104

Table 8. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Neoplastic Nodule or Hepatocellular Carcinoma (b)	5/90(6)	3/50(6)	3/49(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.080	1.102
Lower Limit		0.173	0.177
Upper Limit		5.280	5.383
Weeks to First Observed Tumor	104	104	104
Pituitary: Chromophobe Adenoma (b)	4/84(5)	2/48(4)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.875	1.680
Lower Limit		0.081	0.325
Upper Limit		5.831	8.594
Weeks to First Observed Tumor	104	98	89
Pituitary: Chromophobe Carcinoma (b)	1/84(1)	1/48(2)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.750	5.040
Lower Limit		0.023	0.416
Upper Limit		134.531	259.132
Weeks to First Observed Tumor	104	104	104

Table 8. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma (b)	5/84(6)	3/48(6)	7/50(14)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.050	2.352
Lower Limit		0.169	0.677
Upper Limit		5.121	8.865
Weeks to First Observed Tumor	104	98	89
Adrenal: Pheochromocytoma (b)	11/89(12)	5/49(10)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.826	0.485
Lower Limit		0.236	0.090
Upper Limit		2.402	1.727
Weeks to First Observed Tumor	86	79	89
Preputial Gland: Sebaceous Adenoma or Adenocarcinoma (b)	1/90(1)	0/50(0)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.000	5.400
Lower Limit		0.000	0.446
Upper Limit		33.570	277.604
Weeks to First Observed Tumor	104	--	76

Table 8. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Testis:			
Interstitial-Cell Tumor (b)	86/90 (96)	50/50(100)	47/50(94)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.047	0.984
Lower Limit		0.970	0.900
Upper Limit		1.047	1.060
Weeks to First Observed Tumor	74	65	72
Tunica Vaginalis: Sebaceous			
Adenoma or Adenocarcinoma (b)	1/90(1)	0/50(0)	3/50(6)
P Values (c),(d)	N.S	N.S.	N.S
Relative Risk (Control) (e)		0.000	5.400
Lower Limit		0.000	0.446
Upper Limit		33.570	277.604
Weeks to First Observed Tumor	104	--	76
Tunica Vaginalis, Peritoneum, or			
Multiple Organs: Mesothelioma (b)	3/90(3)	0/50(0)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.000	1.800
Lower Limit		0.000	0.248
Upper Limit		3.000	12.905
Weeks to First Observed Tumor	97	--	104

(a) Dosed groups received doses of 6,000 or 12,500 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table 9. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing C.I. Acid Red 14 (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Lymphocytic Leukemia (b)	16/88(18)	4/50(8)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.440	0.550
Lower Limit		0.112	0.166
Upper Limit		1.270	1.457
Weeks to First Observed Tumor	5	95	93
Hematopoietic System:			
Lymphomas or Leukemias (b)	18/88(20)	4/50(8)	6/50(12)
P Values (c),(d)	N.S.	P=0.043(N)	N.S.
Relative Risk (Control) (e)		0.391	0.587
Lower Limit		0.101	0.202
Upper Limit		1.104	1.423
Weeks to First Observed Tumor	5	95	93
Pituitary:			
Chromophobe Adenoma (b)	25/83(30)	9/46(20)	15/49(31)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.650	1.016
Lower Limit		0.290	0.550
Upper Limit		1.296	1.783
Weeks to First Observed Tumor	81	94	82

Table 9. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary:			
Chromophobe Carcinoma (b)	5/83(6)	0/46(0)	3/49(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.000	1.016
Lower Limit		0.000	0.163
Upper Limit		1.432	4.960
Weeks to First Observed Tumor	104	--	105
Pituitary: Chromophobe			
Adenoma or Carcinoma (b)	30/83(36)	9/46(20)	18/49(37)
P Values (c),(d)	N.S.	P=0.037(N)	N.S.
Departure from Linear Trend (f)	P=0.036		
Relative Risk (Control) (e)		0.541	1.016
Lower Limit		0.247	0.597
Upper Limit		1.050	1.653
Weeks to First Observed Tumor	81	94	82
Adrenal:			
Cortical Adenoma (b)	6/86(7)	3/50(6)	6/50(12)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.860	1.720
Lower Limit		0.144	0.483
Upper Limit		3.816	6.056
Weeks to First Observed Tumor	104	105	88

Table 9. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-Cell			
Carcinoma (b)	3/86(3)	1/49(2)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.585	2.293
Lower Limit		0.011	0.402
Upper Limit		7.016	15.010
Weeks to First Observed Tumor	104	105	101
Mammary Gland:			
Fibroadenoma (b)	18/88(20)	7/50(14)	10/50(20)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.684	0.978
Lower Limit		0.257	0.434
Upper Limit		1.579	2.036
Weeks to First Observed Tumor	81	105	104
Clitoral Gland:			
Sebaceous Adenoma (b)	0/88(0)	0/50(0)	3/50(6)
P Values (c),(d)	P=0.015	N.S.	P=0.046
Relative Risk (Control) (e)		--	Infinite
Lower Limit		--	1.047
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	105

Table 9. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Clitoral Gland:			
Sebaceous Adenoma or Squamous Cell Papilloma or Carcinoma (b)	1/88(1)	1/50(2)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.760	5.280
Lower Limit		0.023	0.436
Upper Limit		135.430	271.440
Weeks to First Observed Tumor	--	--	105
Uterus: Endometrial Stromal Polyp (b)	9/87(10)	11/50(22)	14/50(28)
P Values (c),(d)	P=0.006	N.S.	P=0.008
Relative Risk (Control) (e)		2.127	2.707
Lower Limit		0.857	1.176
Upper Limit		5.359	6.499
Weeks to First Observed Tumor	88	95	100

(a) Dosed groups received doses of 12,500 or 25,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

The mean body weight of the high-dose male mice was slightly lower than that of the controls throughout most of the study; the mean body weight of the high-dose female mice was comparable with that of the controls (Figure 3). No other compound-related clinical signs were observed.

B. Survival (Mice)

Estimates of the probabilities of survival of male and female mice administered C.I. Acid Red 14 in the diet at the concentrations of this bioassay, together with those of the control group, are shown by the Kaplan and Meier curves in Figure 4. There was no significant decrease in survival between any of the groups of male or female mice. The control female mice had lower survival than the dosed groups after 84 weeks.

In female mice, 33/50 (66%) of the control group, 39/50 (78%) of the low-dose group, and 41/50 (82%) of the high-dose group lived to the end of the study at 104 weeks. In male mice, 34/50 (68%) of the control group, 38/50 (76%) of the low-dose group, and 35/50 (70%) of the high-dose group lived to the end of the study at 103-104 weeks.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, Tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, Tables D1 and D2. Each type of tumor

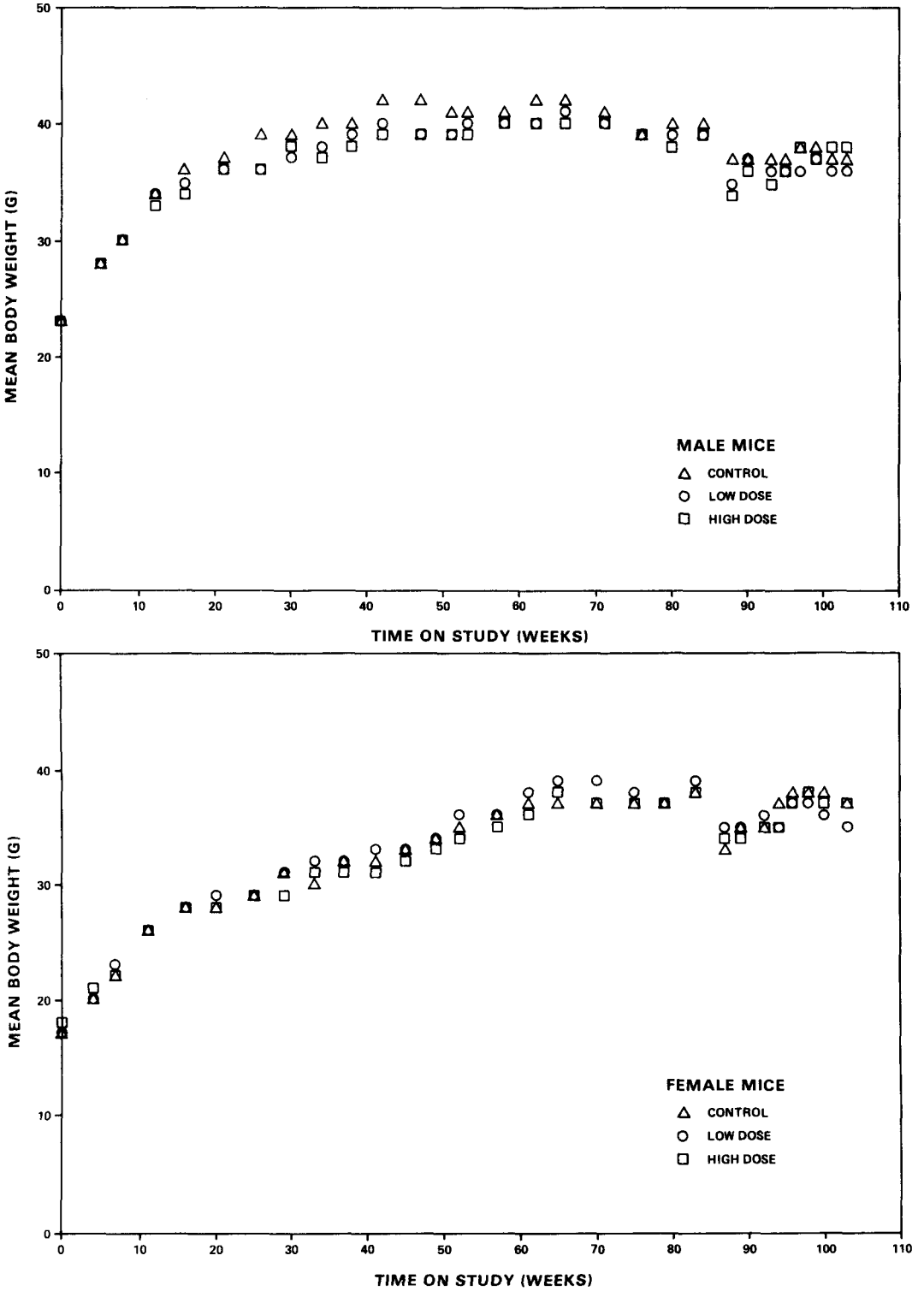


Figure 3. Growth Curves for Mice Fed Diets Containing C.I. Acid Red 14

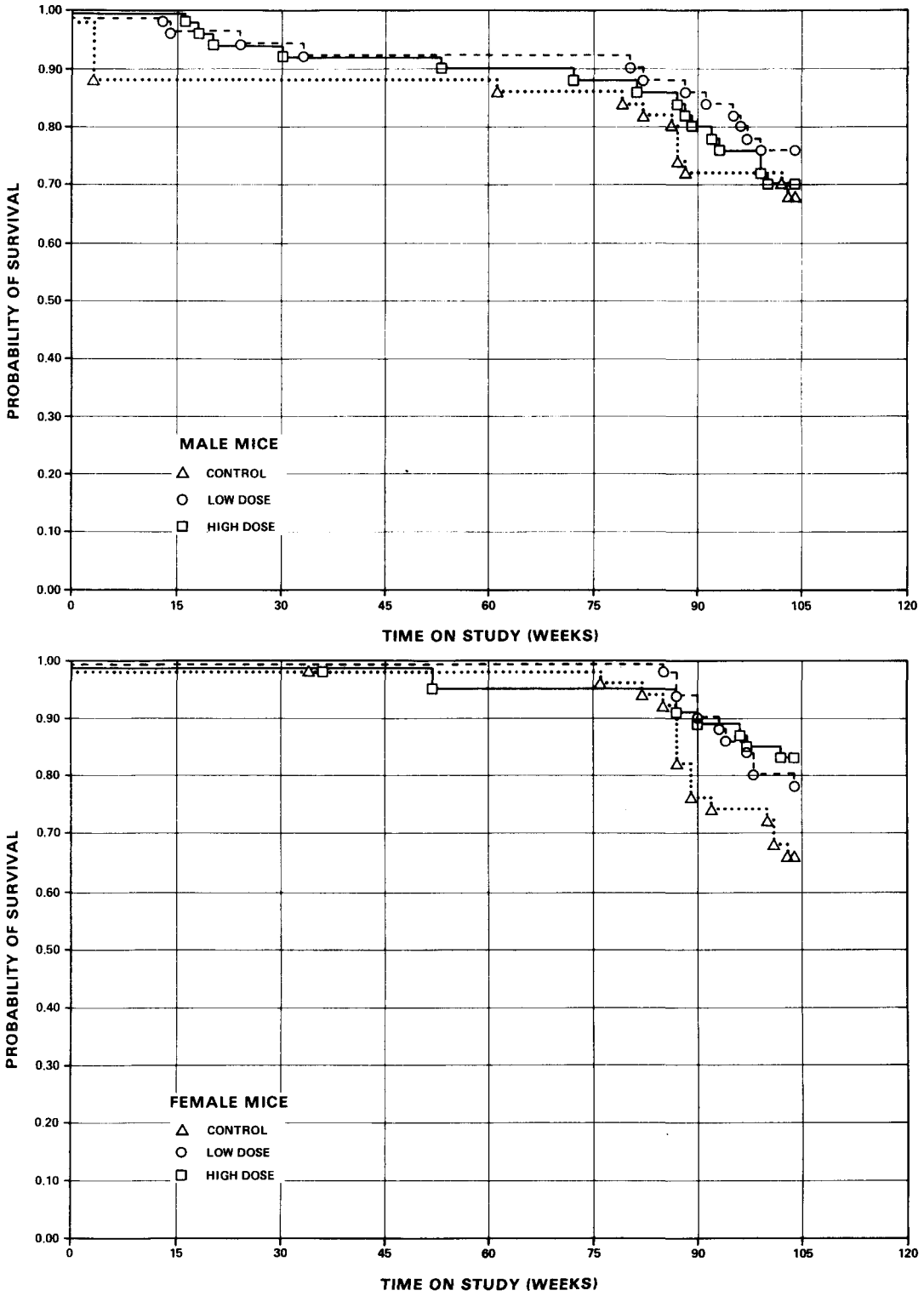


Figure 4. Survival Curves for Mice Fed Diets Containing C.I. Acid Red 14

and the various nonneoplastic lesions represented among both control and dosed animals have been encountered previously as spontaneous occurrences in aging 2-year-old laboratory mice.

Results of histopathologic examination indicated that administration of C.I. Acid Red 14 to male or female B6C3F1 mice under the conditions of the bioassay was not associated with an increased incidence of any tumor type.

D. Statistical Analyses of Results (Mice)

Tables 10 and 11 contain the statistical analyses of those primary tumors that occurred in at least two animals of one group and with an incidence of at least 5% in one or more groups.

Chromophobe adenomas of the pituitary in female mice were observed in increased incidence in the high-dose group (0/36, 0% in the controls; 0/33, 0% in the low-dose; and 3/41, 7% in the high-dose). The Cochran-Armitage test for linear trend was statistically significant in the positive direction (P=0.049). The Fisher exact tests were not significant. In male mice, this tumor was not observed in statistically significant proportions. The historical records at this laboratory indicate this tumor was observed in 3/200 (1.5%) of the female mice.

Malignant lymphomas (histiocytic type) of the hematopoietic system in female mice were observed in decreased incidence in the high-dose group compared with the control group (9/50, 18% in the controls; 10/50, 20% in the low-dose; and 2/48, 4% in the high-dose). The Cochran-Armitage test for linear trend was statistically significant in the negative direction (P=0.036). The Fisher exact test between the high-dose group and the control group indicated a value of P=0.030, but this value is above the value of P=0.025 required by the Bonferroni inequality criterion for an overall significance of P=0.05 when two dosed groups are compared with a

common control group. In male mice, this tumor was not observed in statistically significant proportions.

In the male mice, six of the controls and four in each of the dosed groups died before 52 weeks. The analysis of male mice was repeated, eliminating those animals, and no significant results were observed. Only one of the control group and one of the high-dose female mice were lost to the study before 52 weeks; therefore, time-adjusted tests were not performed. Life table analyses did not produce any additional evidence of carcinogenicity.

The conclusion, based on statistical analysis, is that no increase in tumor incidence at any site is associated with the administration of the chemical.

Table 10. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing C.I. Acid Red 14 (a)

Topography: Morphology	Control	Low Dose	High Dose
Subcutaneous Tissue:			
Fibrosarcoma (b)	3/49(6)	2/50(4)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.653	1.633
Lower Limit		0.057	0.337
Upper Limit		5.457	10.018
Weeks to First Observed Tumor	61	88	93
Lung: Alveolar/Bronchiolar			
Adenoma (b)	4/46(9)	3/49(6)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.704	0.920
Lower Limit		0.109	0.182
Upper Limit		3.940	4.670
Weeks to First Observed Tumor	103	99	88
Lung: Alveolar/Bronchiolar			
Adenoma or Carcinoma (b)	4/46(9)	4/49(8)	4/50(8)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.939	0.920
Lower Limit		0.185	0.182
Upper Limit		4.761	4.670
Weeks to First Observed Tumor	103	99	88

Table 10. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Malignant Lymphoma, Histiocytic Type (b)	1/49(2)	4/50(8)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		3.920	3.920
Lower Limit		0.407	0.407
Upper Limit		188.989	188.989
Weeks to First Observed Tumor	102	80	72
Hematopoietic System:			
Malignant Lymphoma, NOS (b)	3/49(6)	2/50(4)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.653	0.980
Lower Limit		0.057	0.137
Upper Limit		5.457	6.989
Weeks to First Observed Tumor	87	95	100
Hematopoietic System:			
Lymphomas (b)	6/49(12)	7/50(14)	9/50(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.143	1.920
Lower Limit		0.355	0.507
Upper Limit		3.831	4.652
Weeks to First Observed Tumor	86	80	72

Table 10. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing C.I. Acid Red 14 (a) (continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Lymphomas or Leukemias (b)	7/49(14)	7/50(14)	10/50(20)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.980	1.400
Lower Limit		0.317	0.525
Upper Limit		3.032	3.988
Weeks to First Observed Tumor	86	80	72
Liver: Hepatocellular			
Adenoma (b)	6/48(13)	2/50(4)	10/50(20)
P Values (c),(d)	N.S.	N.S.	N.S.
Departure from Linear Trend (f)	P=0.031		
Relative Risk (Control) (e)		0.320	1.600
Lower Limit		0.033	0.574
Upper Limit		1.687	4.953
Weeks to First Observed Tumor	103	104	92
Liver: Hepatocellular			
Carcinoma (b)	10/48(21)	7/50(14)	6/50(12)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.672	0.576
Lower Limit		0.236	0.186
Upper Limit		1.792	1.607
Weeks to First Observed Tumor	103	82	93

Table 10. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma (b)	15/48(31)	9/50(18)	14/50(28)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.576	0.896
Lower Limit		0.247	0.452
Upper Limit		1.264	1.768
Weeks to First Observed Tumor	103	82	93

(a) Dosed groups received doses of 3,000 or 6,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing C.I. Acid Red 14 (a)

Topography: Morphology	Control	Low Dose	High Dose
Subcutaneous Tissue:			
Fibrosarcoma (b)	3/50(6)	2/50(4)	1/48(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.667	0.347
Lower Limit		0.058	0.007
Upper Limit		5.570	4.143
Weeks to First Observed Tumor	85	93	104
Lung: Alveolar/Bronchiolar			
Adenoma (b)	1/50(2)	4/50(8)	3/48(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		4.000	3.125
Lower Limit		0.415	0.262
Upper Limit		192.805	160.536
Weeks to First Observed Tumor	104	87	104
Lung: Alveolar/Bronchiolar			
Carcinoma (b)	3/50(6)	0/50(0)	1/48(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.000	0.347
Lower Limit		0.000	0.007
Upper Limit		1.663	4.143
Weeks to First Observed Tumor	89	--	87

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	4/50(8)	4/50(8)	4/48(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.000	1.042
Lower Limit		0.197	0.205
Upper Limit		5.083	5.286
Weeks to First Observed Tumor	89	87	87
Hematopoietic System: Malignant Lymphoma, Histiocytic Type (b)	9/50(18)	10/50(20)	2/48(4)
P Values (c),(d)	P=0.036(N)	N.S.	P=0.030(N)
Relative Risk (Control) (e)		1.111	0.231
Lower Limit		0.445	0.025
Upper Limit		2.823	1.045
Weeks to First Observed Tumor	82	93	104
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type (b)	2/50(4)	2/50(4)	3/48(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.000	1.563
Lower Limit		0.075	0.187
Upper Limit		13.326	18.028
Weeks to First Observed Tumor	76	85	104

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Malignant Lymphoma, NOS (b)	0/50(0)	4/50(8)	2/48(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		0.927	0.308
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	87
Hematopoietic System:			
Lymphomas (b)	11/50(22)	15/50(30)	8/48(17)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.364	0.758
Lower Limit		0.653	0.289
Upper Limit		2.943	1.882
Weeks to First Observed Tumor	76	85	87
Hematopoietic System:			
Lymphomas or Leukemias (b)	11/50(22)	17/50(34)	8/48(17)
P Values (c),(d)	N.S.	N.S.	N.S.
Departure from Linear Trend (f)	P=0.049		
Relative Risk (Control) (e)		1.545	0.758
Lower Limit		0.765	0.289
Upper Limit		3.257	1.882
Weeks to First Observed Tumor	76	85	87

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Circulatory System:			
Hemangiosarcoma (b)	3/50(6)	1/50(2)	2/48(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.333	0.694
Lower Limit		0.006	0.060
Upper Limit		3.983	5.794
Weeks to First Observed Tumor	101	104	90
Liver:			
Hepatocellular			
Carcinoma (b)	2/50(4)	4/50(8)	1/48(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.000	0.521
Lower Limit		0.301	0.009
Upper Limit		21.316	9.666
Weeks to First Observed Tumor	76	97	104
Liver: Hepatocellular			
Adenoma or Carcinoma (b)	3/50(6)	5/50(10)	2/48(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.667	0.694
Lower Limit		0.344	0.060
Upper Limit		10.225	5.794
Weeks to First Observed Tumor	76	97	104

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing C.I. Acid Red 14 (a)
(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Chromophobe			
Adenoma (b)	0/36(0)	0/33(0)	3/41(7)
P Values (c),(d)	P=0.049	N.S.	N.S.
Relative Risk (Control) (e)		--	Infinite
Lower Limit		--	0.534
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	102

(a) Dosed groups received doses of 3,000 or 6,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

V. DISCUSSION

Mean body weights of dosed rats of either sex and of dosed female mice were comparable with those of the controls. The mean body weight of high-dose male mice was slightly lower than that of the controls. Microscopic renal lesions observed in the subchronic study in male rats fed 25,000 ppm or more and in females fed 100,000 ppm, and splenomegaly in male mice fed 100,000 ppm in the 90-day subchronic study, were not detected in the chronic study.

Survival of low-dose male rats was significantly longer than the survival in the high-dose or control groups. Because there was no appreciable loss in weight gain and because only minimal toxic effects were observed, rats and female mice may have been able to tolerate higher doses in the chronic study. Some nonneoplastic lesions observed in dosed rats of both sexes and in dosed female mice suggest, however, that the doses used approach the maximum tolerated doses. These lesions include focal hyperplasia and lipoidosis of the adrenal cortex in male rats, focal atrophy of the pancreatic acinus in female rats, and lymphoid hyperplasia of the spleen, hematopoiesis in the liver, and lymphoid hyperplasia of the submucosa of the urinary bladder in female mice.

Sebaceous adenomas of the clitoral gland in the female rats occurred in the high-dose group at an incidence significantly higher than that in the controls, but the Bonferroni inequality criterion for comparing two dosed groups with a single control was not met. Furthermore, when adenomas, papillomas and carcinomas of the clitoral gland are combined (high dose 3/50 6%, low dose, 1/50, 2%, control, 1/88 1%) there is no significant difference in incidences and the only carcinoma occurred in the control group.

The true significance of apparent increases in the incidences of clitoral and preputial gland neoplasms is difficult to interpret because only the glands with grossly observable masses were examined microscopically. Small lesions, not easily found during gross examination, could

have been missed. When this sampling technique was used, neoplasms of the clitoral gland were found in 50/2924 (1.7%) control F344 female rats used in the Bioassay Program. However, incidences of neoplasms of the clitoral or preputial glands of 8% to 11% have been reported in rats of the same strain and age (Coleman, et. al., 1977; Reznik and Reznik-Schuller, 1980). Therefore, the apparent increase in the incidences of clitoral gland neoplasms (control, 1/88, 1%; low-dose, 1/50, 2%; high-dose, 3/50, 6%) in this study is not regarded as being associated with compound administration.

Endometrial stromal polyps of the uterus occurred in 28% of the high-dose female rats and at an incidence significantly higher (P=0.008) than that in the controls. However, the observed incidence of this tumor in the dosed groups was similar to the historical control rate at this laboratory (65/286, 23%) thus, the increased incidence of this lesion is not regarded as being associated with the administration of C.I. Acid Red 14.

Since feeding studies on C.I. Acid Orange 10 (NTP, 1982) and on FD&C Yellow No. 6 (NTP, 1981) were conducted in the same room as that on C.I. Acid Red 14, the results from these two experiments are given for comparative uses. Both chemicals were considered not carcinogenic for male and female F344 rats and B6C3F1 mice. Nonetheless, for male mice in the study of FD&C Yellow No. 6, there was a slight increase in the incidence of hepatocellular carcinomas (control: 13/50, 26%; low-dose: 22/48, 46%; high-dose: 16/50, 32%).

Chemical disposition studies were not done on this chemical, and none were located in the literature. Being a water-soluble, highly ionized azo dye, C.I. Acid Red 14 would most likely not be absorbed intact. The potential sulfonic acid metabolites generated from reductive cleavage of the azo linkage in the gastrointestinal tract would probably be absorbed and excreted later via the urine as conjugates. Metabolism studies should be undertaken to clarify the extent of abosorption of C.I. Acid Red 14 or its metabolites.

Although previous *in vitro* mutagenicity studies (Garner and Nutman, 1977; Viola and Nosotti, 1978; and Brown et al., 1978) did not demonstrate a mutagenic effect, the preincubation procedures were oxidative and would be predicted to yield metabolites different from those produced by a system using anaerobic conditions similar to those encountered in the gastrointestinal tract.

The data from this bioassay, coupled with the results of previous long-term studies (Bonser et al., 1956; IARC, 1975; Mason et al., 1974; Holmes et al., 1978), support the finding that C.I. Acid Red 14 is not carcinogenic.

VI. CONCLUSION

Under the conditions of this bioassay, C.I. Acid Red 14 was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED DIETS CONTAINING C.I. ACID RED 14

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED DIETS CONTAINING C.I. ACID RED 14

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	90	50	50
ANIMALS NECROPSIED	90	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	90	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(90)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	3 (3%)		
SEBACEOUS ADENOMA	1 (1%)		
KERATOACANTHOMA			1 (2%)
FIBROMA	1 (1%)		
*SUBCUT TISSUE	(90)	(50)	(50)
FIBROMA	4 (4%)	1 (2%)	1 (2%)
FIBROSARCOMA	1 (1%)		
RESPIRATORY SYSTEM			
#LUNG	(89)	(49)	(50)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (1%)	2 (4%)	1 (2%)
CORTICAL CARCINOMA, METASTATIC		1 (2%)	
PHEOCHROMOCYTOMA, METASTATIC	1 (1%)		
FIBROSARCOMA, METASTATIC	1 (1%)		
OSTEOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(90)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (1%)		
LYMPHOCYTIC LEUKEMIA	21 (23%)	6 (12%)	11 (22%)
#BONE MARROW	(84)	(49)	(47)
OSTEOMA	1 (1%)		
#SPLEEN	(90)	(50)	(49)
ISLET-CELL CARCINOMA, METASTATIC			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
LYMPHOCYTIC LEUKEMIA	1 (1%)	1 (2%)	
#MANDIBULAR L. NODE	(89)	(48)	(44)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#LYMPH NODE OF THORAX	(89)	(48)	(44)
INTERSTITIAL-CELL TUMOR, METASTA	1 (1%)		
#MESENTERIC L. NODE	(89)	(48)	(44)
MUCINOUS ADENOCARCINOMA, METASTA	1 (1%)		
LEIOMYOSARCOMA, METASTATIC			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(90)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIV	1 (1%)		
NONCHROMAFFIN PARAGANGLIOMA	1 (1%)		
NEURILEMOMA, MALIGNANT		1 (2%)	1 (2%)
DIGESTIVE SYSTEM			
*INTESTINAL TRACT	(90)	(50)	(50)
MUCINOUS ADENOCARCINOMA	1 (1%)		
LEIOMYOSARCOMA			1 (2%)
#SALIVARY GLAND	(89)	(50)	(50)
MIXED TUMOR, MALIGNANT	1 (1%)		
#LIVER	(90)	(50)	(49)
NEOPLASTIC NODULE	5 (6%)	1 (2%)	2 (4%)
HEPATOCELLULAR CARCINOMA		2 (4%)	2 (4%)
FIBROSARCOMA, METASTATIC	1 (1%)		
#PANCREAS	(88)	(47)	(49)
CORTICAL CARCINOMA, METASTATIC		1 (2%)	
LEIOMYOSARCOMA, INVASIVE			2 (4%)
#GASTRIC SEROSA	(87)	(50)	(48)
NEOPLASM, NOS			1 (2%)
#CARDIAC STOMACH	(87)	(50)	(48)
SQUAMOUS CELL PAPILLOMA	1 (1%)		
#LARGE INTESTINE	(87)	(47)	(46)
LEIOMYOSARCOMA			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#COLON	(87)	(47)	(46)
ADENOMATOUS POLYP, NOS	1 (1%)		
URINARY SYSTEM			
#KIDNEY	(90)	(50)	(50)
TUBULAR-CELL ADENOMA		1 (2%)	
CORTICAL CARCINOMA, METASTATIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(84)	(48)	(50)
ADENOMA, NOS	1 (1%)		
CHROMOPHOBE ADENOMA	4 (5%)	2 (4%)	4 (8%)
CHROMOPHOBE CARCINOMA	1 (1%)	1 (2%)	3 (6%)
ACIDOPHIL ADENOMA	2 (2%)		
#ADRENAL	(89)	(49)	(50)
CORTICAL ADENOMA	3 (3%)	1 (2%)	1 (2%)
CORTICAL CARCINOMA		1 (2%)	1 (2%)
PHEOCHROMOCYTOMA	11 (12%)	5 (10%)	3 (6%)
PHEOCHROMOCYTOMA, MALIGNANT	3 (3%)		
#THYROID	(89)	(49)	(50)
FOLLICULAR-CELL ADENOMA	2 (2%)		1 (2%)
FOLLICULAR-CELL CARCINOMA	2 (2%)		
C-CELL CARCINOMA	2 (2%)	1 (2%)	2 (4%)
#PANCREATIC ISLETS	(88)	(47)	(49)
ISLET-CELL CARCINOMA	3 (3%)	1 (2%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(90)	(50)	(50)
FIBROADENOMA	2 (2%)	1 (2%)	1 (2%)
*PREPUTIAL GLAND	(90)	(50)	(50)
SEBACEOUS ADENOMA			1 (2%)
SEBACEOUS ADENOCARCINOMA	1 (1%)		2 (4%)
#TESTIS	(90)	(50)	(50)
CORTICAL CARCINOMA, METASTATIC		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INTERSTITIAL-CELL TUMOR	86 (96%)	50 (100%)	47 (94%)
INTERSTITIAL-CELL TUMOR, MALIGNA	1 (1%)		
NERVOUS SYSTEM			
#CEREBRUM	(90)	(50)	(50)
ASTROCYTOMA	1 (1%)		
#BRAIN	(90)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
CHROMOPHOBE CARCINOMA, INVASIVE			1 (2%)
ASTROCYTOMA	1 (1%)	1 (2%)	
OLIGODENDROGLIOMA	1 (1%)		
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(90)	(50)	(50)
SEBACEOUS ADENOCARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
*FEMUR	(90)	(50)	(50)
OSTEOSARCOMA			1 (2%)
*INTERCOSTAL MUSCLE	(90)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIV	1 (1%)		
BODY CAVITIES			
*PERITONEUM	(90)	(50)	(50)
MESOTHELIOMA, MALIGNANT	1 (1%)		
*MESENTERY	(90)	(50)	(50)
LEIOMYOSARCOMA, INVASIVE			2 (4%)
*TUNICA VAGINALIS	(90)	(50)	(50)
CORTICAL CARCINOMA, METASTATIC		1 (2%)	
MESOTHELIOMA, NOS			3 (6%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(90)	(50)	(50)
MESOTHELIOMA, NOS	1 (1%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MESOTHELIOMA, MALIGNANT	1 (1%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	90	50	50
NATURAL DEATH@	9	7	12
MORIBUND SACRIFICE	11	1	6
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	70	28	30
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS		14	2
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	90	50	49
TOTAL PRIMARY TUMORS	175	79	95
TOTAL ANIMALS WITH BENIGN TUMORS	86	50	48
TOTAL BENIGN TUMORS	124	61	60
TOTAL ANIMALS WITH MALIGNANT TUMORS	40	13	27
TOTAL MALIGNANT TUMORS	45	17	29
TOTAL ANIMALS WITH SECONDARY TUMORS#	4	3	5
TOTAL SECONDARY TUMORS	7	8	8
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	6	1	6
TOTAL UNCERTAIN TUMORS	6	1	6
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED DIETS CONTAINING C.I. ACID RED 14

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	90	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	88	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	88	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(88)	(50)	(50)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
BASAL-CELL CARCINOMA			1 (2%)
*SUBCUT TISSUE	(88)	(50)	(50)
FIBROMA	2 (2%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(88)	(48)	(50)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
CORTICAL CARCINOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(88)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (2%)		
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
LYMPHOCYTIC LEUKEMIA	14 (16%)	4 (8%)	5 (10%)
#SPLEEN	(88)	(50)	(50)
LYMPHOCYTIC LEUKEMIA	2 (2%)		
#BRONCHIAL LYMPH NODE	(86)	(43)	(45)
CORTICAL CARCINOMA, METASTATIC			1 (2%)
#RENAL LYMPH NODE	(86)	(43)	(45)
TRANSITIONAL-CELL CARCINOMA, MET	1 (1%)		
CIRCULATORY SYSTEM			
#SPLEEN	(88)	(50)	(50)
ANGIOSARCOMA			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#BRONCHIAL LYMPH NODE	(86)	(43)	(45)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
#LUNG	(88)	(48)	(50)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
*PLEURA	(88)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
*MEDIASTINAL PLEURA	(88)	(50)	(50)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
#HEART	(88)	(49)	(48)
NEURILEMOMA, MALIGNANT	1 (1%)		
DIGESTIVE SYSTEM			
#LIVER	(88)	(50)	(50)
NEOPLASTIC NODULE	3 (3%)	1 (2%)	1 (2%)
#JEJUNUM	(85)	(48)	(48)
FIBROSARCOMA		1 (2%)	
#ILEUM	(85)	(48)	(48)
FIBROSARCOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY/PELVIS	(88)	(50)	(50)
TRANSITIONAL-CELL CARCINOMA	1 (1%)		
#URINARY BLADDER	(80)	(45)	(48)
LEIOMYOSARCOMA, INVASIVE			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(83)	(46)	(49)
CHROMOPHOBE ADENOMA	25 (30%)	9 (20%)	15 (31%)
CHROMOPHOBE CARCINOMA	5 (6%)		3 (6%)
ACIDOPHIL ADENOMA		1 (2%)	
ACIDOPHIL CARCINOMA		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#ADRENAL	(86)	(50)	(50)
CORTICAL ADENOMA	6 (7%)	3 (6%)	6 (12%)
CORTICAL CARCINOMA	1 (1%)		1 (2%)
PHEOCHROMOCYTOMA	3 (3%)	1 (2%)	2 (4%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (1%)	1 (2%)	
#THYROID	(86)	(49)	(50)
FOLLICULAR-CELL ADENOMA	1 (1%)		1 (2%)
C-CELL CARCINOMA	3 (3%)	1 (2%)	4 (8%)
#PANCREATIC ISLETS	(83)	(48)	(50)
ISLET-CELL CARCINOMA	1 (1%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(88)	(50)	(50)
ADENOMA, NOS	2 (2%)	1 (2%)	2 (4%)
ADENOCARCINOMA, NOS	2 (2%)		1 (2%)
CYSTADENOMA, NOS			1 (2%)
ACINAR-CELL ADENOMA		1 (2%)	
FIBROADENOMA	18 (20%)	7 (14%)	10 (20%)
*CLITORAL GLAND	(88)	(50)	(50)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
SEBACEOUS ADENOMA			3 (6%)
SQUAMOUS CELL CARCINOMA	1 (1%)		
*VAGINA	(88)	(50)	(50)
FIBROMA	1 (1%)		
#UTERUS	(87)	(50)	(50)
LEIOMYOSARCOMA		2 (4%)	1 (2%)
ENDOMETRIAL STROMAL POLYP	9 (10%)	11 (22%)	14 (28%)
NERVOUS SYSTEM			
#BRAIN	(88)	(50)	(50)
CHROMOPHOBE CARCINOMA, INVASIVE			1 (2%)
EPENDYMOMA	1 (1%)		
ASTROCYTOMA		1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(88)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(88)	(50)	(50)
CORTICAL CARCINOMA, METASTATIC			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(88)	(50)	(50)
SARCOMA, NOS	1 (1%)		
FACE			
SQUAMOUS CELL CARCINOMA		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	90	50	50
NATURAL DEATH@	11	4	8
MORIBUND SACRIFICE	11	5	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	66	41	38
ANIMAL MISSING	2		

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	68	34	44
TOTAL PRIMARY TUMORS	106	52	77
TOTAL ANIMALS WITH BENIGN TUMORS	50	26	32
TOTAL BENIGN TUMORS	67	37	54
TOTAL ANIMALS WITH MALIGNANT TUMORS	33	13	19
TOTAL MALIGNANT TUMORS	36	14	22
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	4
TOTAL SECONDARY TUMORS	1	3	6
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3	1	1
TOTAL UNCERTAIN TUMORS	3	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED DIETS CONTAINING C.I. ACID RED 14

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED DIETS CONTAINING C.I. ACID RED 14

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	48	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
FIBROMA			1 (2%)
FIBROSARCOMA			1 (2%)
*SUBCUT TISSUE	(49)	(50)	(50)
SARCOMA, NOS	1 (2%)	1 (2%)	2 (4%)
FIBROMA			2 (4%)
FIBROSARCOMA	3 (6%)	2 (4%)	5 (10%)
OSTEOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(46)	(49)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)	2 (4%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (9%)	3 (6%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
SARCOMA, NOS, METASTATIC		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (2%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (2%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	2 (4%)	2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	1 (2%)
LYMPHOCYTIC LEUKEMIA	1 (2%)		1 (2%)
#SPLEEN	(45)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)		3 (6%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LYMPH NODE	(36)	(39)	(38)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (5%)	
#BRONCHIAL LYMPH NODE	(36)	(39)	(38)
MALIGNANT LYMPHOMA, NOS	1 (3%)		
#MESENTERIC L. NODE	(36)	(39)	(38)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			2 (5%)
#PEYER'S PATCH	(40)	(49)	(45)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE	1 (3%)		
CIRCULATORY SYSTEM			
#BONE MARROW	(46)	(49)	(46)
ANGIOSARCOMA, METASTATIC		1 (2%)	
#SPLEEN	(45)	(49)	(50)
ANGIOSARCOMA		1 (2%)	
#LIVER	(48)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
ANGIOSARCOMA	2 (4%)	1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(48)	(50)	(50)
BILE DUCT CARCINOMA		1 (2%)	
HEPATOCELLULAR ADENOMA	6 (13%)	2 (4%)	10 (20%)
HEPATOCELLULAR CARCINOMA	10 (21%)	7 (14%)	6 (12%)
SARCOMA, NOS		1 (2%)	
#STOMACH	(44)	(49)	(46)
ADENOMA, NOS			2 (4%)
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#ADRENAL	(46)	(47)	(46)
CORTICAL ADENOMA	2 (4%)	1 (2%)	2 (4%)
#ADRENAL/CAPSULE	(46)	(47)	(46)
ADENOMA, NOS	2 (4%)		
#THYROID	(44)	(49)	(44)
FOLLICULAR-CELL ADENOMA		2 (4%)	
PAPILLARY CYSTADENOMA, NOS			1 (2%)
#PANCREATIC ISLETS	(46)	(48)	(48)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
#TESTIS	(47)	(50)	(49)
INTERSTITIAL-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(49)	(50)	(50)
SARCOMA, NOS, METASTATIC			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(49)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
FIBROSARCOMA, METASTATIC	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	15	10	13
MORIBUND SACRIFICE	1	2	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	34	38	35
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	31	28	31
TOTAL PRIMARY TUMORS	38	33	47
TOTAL ANIMALS WITH BENIGN TUMORS	13	9	19
TOTAL BENIGN TUMORS	15	9	22
TOTAL ANIMALS WITH MALIGNANT TUMORS	21	21	21
TOTAL MALIGNANT TUMORS	23	24	25
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	5	2
TOTAL SECONDARY TUMORS	3	5	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED DIETS CONTAINING C.I. ACID RED 14

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(48)
FIBROSARCOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(48)
FIBROSARCOMA	3 (6%)	2 (4%)	1 (2%)
OSTEOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(48)
ADENOCARCINOMA, NOS, METASTATIC	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	4 (8%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (6%)		1 (2%)
ADENOSQUAMOUS CARCINOMA, METASTA		1 (2%)	
OSTEOSARCOMA, METASTATIC	1 (2%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(48)
MALIGNANT LYMPHOMA, NOS		3 (6%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (4%)	1 (2%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	9 (18%)	8 (16%)	2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	1 (2%)
LYMPHOCYTIC LEUKEMIA		2 (4%)	
#SPLEEN	(48)	(50)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#LYMPH NODE	(41)	(38)	(37)
MALIGNANT LYMPHOMA, NOS			1 (3%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MANDIBULAR L. NODE	(41)	(38)	(37)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)
#PANCREATIC L.NODE	(41)	(38)	(37)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
#PEYER'S PATCH	(44)	(47)	(45)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#THYMUS	(35)	(39)	(42)
OSTEOSARCOMA, METASTATIC	1 (3%)		
MALIGNANT LYMPHOMA, NOS		1 (3%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(48)
HEMANGIOSARCOMA	2 (4%)		
#SPLEEN	(48)	(50)	(48)
HEMANGIOSARCOMA		1 (2%)	1 (2%)
#LIVER	(50)	(50)	(48)
HEMANGIOSARCOMA	1 (2%)		1 (2%)
ANGIOSARCOMA	1 (2%)		
#UTERUS	(49)	(49)	(48)
HEMANGIOMA			1 (2%)
#OVARY	(45)	(47)	(44)
HEMANGIOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(48)
HEPATOCELLULAR ADENOMA	1 (2%)	1 (2%)	1 (2%)
HEPATOCELLULAR CARCINOMA	2 (4%)	4 (8%)	1 (2%)
OSTEOSARCOMA, METASTATIC	1 (2%)		
#CARDIAC STOMACH	(50)	(48)	(48)
SQUAMOUS CELL PAPILLOMA			1 (2%)
URINARY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(36)	(33)	(41)
ADENOMA, NOS	1 (3%)		
CHROMOPHOBE ADENOMA			3 (7%)
#ADRENAL	(46)	(48)	(48)
CORTICAL ADENOMA	1 (2%)		
PHEOCHROMOCYTOMA	1 (2%)		
PHEOCHROMOCYTOMA, MALIGNANT		1 (2%)	
#ADRENAL/CAPSULE	(46)	(48)	(48)
ADENOMA, NOS	1 (2%)		
#THYROID	(48)	(49)	(48)
FOLLICULAR-CELL ADENOMA	1 (2%)	2 (4%)	1 (2%)
FOLLICULAR-CELL CARCINOMA			1 (2%)
#THYROID FOLLICLE	(48)	(49)	(48)
CYSTADENOMA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(48)
ADENOCARCINOMA, NOS			1 (2%)
ACINAR-CELL ADENOMA	1 (2%)		
ADENOSQUAMOUS CARCINOMA		1 (2%)	
#UTERUS	(49)	(49)	(48)
LEIOMYOSARCOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP			2 (4%)
#OVARY	(45)	(47)	(44)
PAPILLARY ADENOMA	2 (4%)		1 (2%)
LUTEOMA			1 (2%)
GRANULOSA-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(50)	(50)	(48)
ADENOMA, NOS	1 (2%)	1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ADENOCARCINOMA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
THORACIC CAVITY			
OSTEOSARCOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATHS	13	9	6
MORIBUND SACRIFICE	4	2	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	33	39	41
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	28	29	23
TOTAL PRIMARY TUMORS	37	39	32
TOTAL ANIMALS WITH BENIGN TUMORS	10	7	13
TOTAL BENIGN TUMORS	11	8	17
TOTAL ANIMALS WITH MALIGNANT TUMORS	20	25	13
TOTAL MALIGNANT TUMORS	26	30	15
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	2	
TOTAL SECONDARY TUMORS	4	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED DIETS CONTAINING C.I. ACID RED 14

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED DIETS CONTAINING C.I. ACID RED 14

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	90	50	50
ANIMALS NECROPSIED	90	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	90	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(90)	(50)	(50)
EPIDERMAL INCLUSION CYST	2 (2%)		2 (4%)
EROSION			1 (2%)
*SUBCUT TISSUE	(90)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)
ABSCESS, CHRONIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(89)	(49)	(50)
HYPERPLASIA, EPITHELIAL		1 (2%)	
#LUNG	(89)	(49)	(50)
MUCOCELE		1 (2%)	
EDEMA, NOS		9 (18%)	3 (6%)
HEMORRHAGE	1 (1%)		
INFLAMMATION, INTERSTITIAL	2 (2%)		1 (2%)
PNEUMONIA, ASPIRATION			1 (2%)
INFLAMMATION, ACUTE NECROTIZING			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
NECROSIS, FOCAL	1 (1%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (1%)		1 (2%)
METAPLASIA, SQUAMOUS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(84)	(49)	(47)
CONGESTION, ACUTE	1 (1%)		
FIBROSIS, FOCAL	1 (1%)		
HYPERPLASIA, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MYELOFIBROSIS		1 (2%)	
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPOPLASIA, HEMATOPOIETIC	4 (5%)	1 (2%)	
#SPLEEN	(90)	(50)	(49)
CONGESTION, NOS	1 (1%)		
INFLAMMATION, NECRO GRAN		1 (2%)	
FIBROSIS, DIFFUSE	1 (1%)		
INFARCT, FOCAL	1 (1%)		
HEMOSIDEROSIS		1 (2%)	
LYMPHOID DEPLETION		1 (2%)	2 (4%)
#SPLENIC CAPSULE	(90)	(50)	(49)
HEMORRHAGE			1 (2%)
#SPLENIC RED PULP	(90)	(50)	(49)
HEMOSIDEROSIS			1 (2%)
#LYMPH NODE	(89)	(48)	(44)
EDEMA, NOS	1 (1%)		1 (2%)
LYMPHOID DEPLETION	1 (1%)		
#SUBMANDIBULAR L.NODE	(89)	(48)	(44)
HEMORRHAGE	1 (1%)		
#MANDIBULAR L. NODE	(89)	(48)	(44)
ABSCESS, CHRONIC			1 (2%)
#MESENTERIC L. NODE	(89)	(48)	(44)
LYMPHOID DEPLETION	1 (1%)		
#LUNG	(89)	(49)	(50)
HYPERPLASIA, LYMPHOID	1 (1%)		
#THYMUS	(69)	(36)	(28)
ATROPHY, DIFFUSE			1 (4%)
HYPERPLASIA, EPITHELIAL			1 (4%)
CIRCULATORY SYSTEM			
#MESENTERIC L. NODE	(89)	(48)	(44)
LYMPHANGIECTASIS			1 (2%)
#HEART	(90)	(50)	(50)
MINERALIZATION	1 (1%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
PERIARTERITIS		1 (2%)	
ENDOCARDIOSIS	1 (1%)		
HYPERPLASIA, FOCAL			1 (2%)
#HEART/ATRIUM	(90)	(50)	(50)
THROMBOSIS, NOS	1 (1%)	1 (2%)	2 (4%)
THROMBUS, ORGANIZED		1 (2%)	
#LEFT ATRIUM	(90)	(50)	(50)
THROMBOSIS, NOS	1 (1%)		
#MYOCARDIUM	(90)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC	1 (1%)		1 (2%)
FIBROSIS, FOCAL	1 (1%)		
DEGENERATION, NOS	31 (34%)	26 (52%)	24 (48%)
#ENDOCARDIUM	(90)	(50)	(50)
INFLAMMATION PROLIFERATIVE		1 (2%)	
#CARDIAC VALVE	(90)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (1%)		
FIBROSIS	1 (1%)		
FIBROSIS, FOCAL	1 (1%)		
FIBROSIS, DIFFUSE			1 (2%)
*PULMONARY ARTERY	(90)	(50)	(50)
MINERALIZATION		1 (2%)	
*PANCREATIC ARTERY	(90)	(50)	(50)
MINERALIZATION		1 (2%)	
FIBROSIS	1 (1%)	1 (2%)	
*MESENTERIC ARTERY	(90)	(50)	(50)
THROMBOSIS, NOS	1 (1%)		
INFLAMMATION, ACUTE/CHRONIC	1 (1%)		
#LIVER	(90)	(50)	(49)
THROMBOSIS, NOS	1 (1%)		
*MESENTERY	(90)	(50)	(50)
PERIARTERITIS		1 (2%)	
#KIDNEY	(90)	(50)	(50)
PERIARTERITIS			1 (2%)
#U.BLADDER/SEROSA	(82)	(44)	(43)
PERIARTERITIS	1 (1%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(89)	(50)	(50)
CYTOPLASMIC CHANGE, NOS		1 (2%)	
ATROPHY, FOCAL	2 (2%)		1 (2%)
#LIVER	(90)	(50)	(49)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
INFLAMMATION, CHRONIC FOCAL	2 (2%)		
GRANULOMA, NOS		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU	1 (1%)		
CIRRHOSIS, NOS	1 (1%)		
DEGENERATION, NOS	1 (1%)		1 (2%)
NECROSIS, FOCAL		1 (2%)	
LIPOIDOSIS			1 (2%)
BASOPHILIC CYTO CHANGE	63 (70%)	28 (56%)	26 (53%)
FOCAL CELLULAR CHANGE	2 (2%)	5 (10%)	4 (8%)
ANGIECTASIS			3 (6%)
#PORTA HEPATIS	(90)	(50)	(49)
FIBROSIS	1 (1%)		
#PORTAL TRACT	(90)	(50)	(49)
FIBROSIS, FOCAL			1 (2%)
#LIVER/CENTRILOBULAR	(90)	(50)	(49)
DEGENERATION, NOS	1 (1%)	1 (2%)	2 (4%)
NECROSIS, FOCAL	1 (1%)	2 (4%)	4 (8%)
#BILE DUCT	(90)	(50)	(49)
HYPERPLASIA, NOS	7 (8%)	2 (4%)	1 (2%)
HYPERPLASIA, FOCAL	15 (17%)	9 (18%)	8 (16%)
#PANCREATIC ACINUS	(88)	(47)	(49)
ATROPHY, NOS	2 (2%)		1 (2%)
ATROPHY, FOCAL	12 (14%)	10 (21%)	3 (6%)
HYPERPLASIA, FOCAL		1 (2%)	
#STOMACH	(87)	(50)	(48)
ULCER, ACUTE		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE FOCAL	1 (1%)		
ULCER, CHRONIC			1 (2%)
HYPERPLASIA, BASAL CELL	1 (1%)		
#GASTRIC MUCOSA	(87)	(50)	(48)
MINERALIZATION	1 (1%)		
NECROSIS, FOCAL	2 (2%)		1 (2%)
#GASTRIC SUBMUCOSA	(87)	(50)	(48)
EDEMA, NOS			1 (2%)
#CARDIAC STOMACH	(87)	(50)	(48)
ECTOPIA	1 (1%)		
HYPERPLASIA, BASAL CELL	1 (1%)		
ACANTHOSIS			1 (2%)
#COLON	(87)	(47)	(46)
NEMATODIASIS	8 (9%)	4 (9%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(90)	(50)	(50)
CYST, NOS	1 (1%)		1 (2%)
ABSCESS, CHRONIC		1 (2%)	
NEPHROPATHY	80 (89%)	44 (88%)	41 (82%)
DEGENERATION, HYALINE	1 (1%)		
GLOMERULOSCLEROSIS, NOS			1 (2%)
PIGMENTATION, NOS	4 (4%)	1 (2%)	
ANGIECTASIS		1 (2%)	
#KIDNEY/CORTEX	(90)	(50)	(50)
NEPHROSIS, NOS			1 (2%)
PIGMENTATION, NOS	2 (2%)		
#KIDNEY/TUBULE	(90)	(50)	(50)
PIGMENTATION, NOS	1 (1%)		2 (4%)
REGENERATION, NOS	1 (1%)		
#KIDNEY/PELVIS	(90)	(50)	(50)
INFLAMMATION, ACUTE FOCAL			1 (2%)
HYPERPLASIA, EPITHELIAL		1 (2%)	1 (2%)
*PROSTATIC URETHRA	(90)	(50)	(50)
METAPLASIA, SQUAMOUS	1 (1%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(84)	(48)	(50)
ULTIMOBRANCHIAL CYST		1 (2%)	
HYPERPLASIA, FOCAL	1 (1%)		
HYPERPLASIA, CHROMOPHOBE-CELL	9 (11%)	2 (4%)	3 (6%)
ANGIECTASIS		1 (2%)	
#PITUITARY ACIDOPHIL	(84)	(48)	(50)
HYPERPLASIA, FOCAL	1 (1%)		1 (2%)
#PITUITARY/BASOPHIL	(84)	(48)	(50)
HYPERPLASIA, FOCAL		2 (4%)	1 (2%)
#ADRENAL	(89)	(49)	(50)
FOCAL CELLULAR CHANGE		1 (2%)	
#ADRENAL CORTEX	(89)	(49)	(50)
LIPOIDOSIS	3 (3%)	2 (4%)	9 (18%)
CYTOPLASMIC VACUOLIZATION	1 (1%)		
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASIA, FOCAL	5 (6%)	6 (12%)	8 (16%)
#ADRENAL MEDULLA	(89)	(49)	(50)
HYPERPLASIA, NOS			2 (4%)
HYPERPLASIA, FOCAL	4 (4%)	7 (14%)	3 (6%)
ANGIECTASIS	1 (1%)		
#THYROID	(89)	(49)	(50)
MINERALIZATION	1 (1%)		
HYPERPLASIA, C-CELL	17 (19%)	5 (10%)	9 (18%)
#PARATHYROID	(70)	(37)	(39)
HYPERPLASIA, NOS		1 (3%)	
#PANCREATIC ISLETS	(88)	(47)	(49)
HYPERPLASIA, NOS	2 (2%)		
HYPERPLASIA, FOCAL	3 (3%)	5 (11%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(90)	(50)	(50)
DILATATION, NOS	1 (1%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASIA, CYSTIC	1 (1%)		1 (2%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
*MAMMARY ACINUS	(90)	(50)	(50)
DILATATION, NOS			1 (2%)
CYST, NOS			1 (2%)
HYPERPLASIA, NOS	5 (6%)		
HYPERPLASIA, CYSTIC			2 (4%)
*PREPUTIAL GLAND	(90)	(50)	(50)
CYST, NOS	1 (1%)		1 (2%)
#PROSTATE	(84)	(46)	(44)
INFLAMMATION, SUPPURATIVE	1 (1%)		
INFLAMMATION, ACUTE FOCAL		2 (4%)	1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)
INFLAMMATION ACTIVE CHRONIC			1 (2%)
INFLAMMATION, ACUTE/CHRONIC	2 (2%)		
INFLAMMATION, FOCAL GRANULOMATOU	1 (1%)		
#PROSTATIC GLAND	(84)	(46)	(44)
DILATATION, NOS	1 (1%)		
HYPERPLASIA, EPITHELIAL	1 (1%)		
*SEMINAL VESICLE	(90)	(50)	(50)
CYST, NOS	1 (1%)		
#TESTIS	(90)	(50)	(50)
STEATITIS	4 (4%)		
ATROPHY, NOS	1 (1%)	1 (2%)	1 (2%)
HYPOSPERMATOGENESIS	1 (1%)		
HYPERPLASIA, INTERSTITIAL CELL		2 (4%)	3 (6%)
#TESTIS/TUBULE	(90)	(50)	(50)
ATROPHY, DIFFUSE			1 (2%)
*EPIDIDYMIS	(90)	(50)	(50)
GRANULOMA, SPERMATIC		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(90)	(50)	(50)
HYDROCEPHALUS, NOS		1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEMORRHAGE	1 (1%)	2 (4%)	1 (2%)
ABSCESS, NOS			1 (2%)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL	1 (1%)		1 (2%)
ATROPHY, PRESSURE		1 (2%)	1 (2%)
#HIPPOCAMPUS	(90)	(50)	(50)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL	1 (1%)		
#HYPOTHALAMUS	(90)	(50)	(50)
ATROPHY, PRESSURE			2 (4%)
#CEREBELLUM	(90)	(50)	(50)
HEMORRHAGE			1 (2%)
NECROSIS, HEMORRHAGIC	1 (1%)		
#MEDULLA OBLONGATA	(90)	(50)	(50)
NECROSIS, HEMORRHAGIC	1 (1%)		
SPECIAL SENSE ORGANS			
*EYE	(90)	(50)	(50)
SYNECHIA, POSTERIOR	1 (1%)		
*EYE/RETINA	(90)	(50)	(50)
DETACHMENT	1 (1%)		
*EYE/CRYSTALLINE LENS	(90)	(50)	(50)
DEGENERATION, NOS	1 (1%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(90)	(50)	(50)
STEATITIS		1 (2%)	
NECROSIS, FAT		1 (2%)	
*PERITONEUM	(90)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC FOCAL	1 (1%)		
*MESENTERY	(90)	(50)	(50)
STEATITIS	4 (4%)		
GRANULOMA, NOS		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU		4 (8%)	1 (2%)
NECROSIS, FAT		3 (6%)	
ALL OTHER SYSTEMS			
CRANIOBUCCAL POUCH			
CYSTIC DUCTS	1		1
SPECIAL MORPHOLOGY SUMMARY			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED DIETS CONTAINING C.I. ACID RED 14

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	90	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	88	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	88	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(88)	(50)	(50)
INFLAMMATION, ACUTE FOCAL			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
*SUBCUT TISSUE	(88)	(50)	(50)
ABSCESS, CHRONIC	1 (1%)		
RESPIRATORY SYSTEM			
#PERITRACHEAL TISSUE	(86)	(47)	(50)
INFLAMMATION, CHRONIC	1 (1%)		
#LUNG	(88)	(48)	(50)
EDEMA, NOS	2 (2%)		
HEMORRHAGE	1 (1%)		
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
PNEUMONIA INTERSTITIAL CHRONIC	1 (1%)		
HEMOSIDEROSIS		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(86)	(50)	(49)
DEPLETION	1 (1%)		
HYPERPLASIA, RETICULUM CELL	2 (2%)	6 (12%)	3 (6%)
HYPOPLASIA, HEMATOPOIETIC	6 (7%)	1 (2%)	
#SPLEEN	(88)	(50)	(50)
CONGESTION, NOS	1 (1%)	5 (10%)	1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU	1 (1%)		
FIBROSIS, FOCAL	1 (1%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFARCT, FOCAL	1 (1%)		
HEMOSIDEROSIS		2 (4%)	
LYMPHOID DEPLETION	2 (2%)		
HEMATOPOIESIS	1 (1%)		1 (2%)
#LYMPH NODE	(86)	(43)	(45)
EDEMA, NOS		1 (2%)	
GRANULOMA, NOS		1 (2%)	
LYMPHOID DEPLETION	1 (1%)		
#SUBMANDIBULAR L.NODE	(86)	(43)	(45)
EDEMA, NOS		1 (2%)	
#MANDIBULAR L. NODE	(86)	(43)	(45)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (1%)		
PLASMACYTOSIS	1 (1%)		
#LYMPH NODE OF THORAX	(86)	(43)	(45)
GRANULOMA, NOS		2 (5%)	
#MESENTERIC L. NODE	(86)	(43)	(45)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU			2 (4%)
LYMPHOID DEPLETION	1 (1%)		
PLASMACYTOSIS	1 (1%)		
#LUNG	(88)	(48)	(50)
LEUKOCYTOSIS, NEUTROPHILIC			1 (2%)
HYPERPLASIA, LYMPHOID	1 (1%)		
#THYMUS	(70)	(39)	(39)
CYST, NOS			1 (3%)
CIRCULATORY SYSTEM			
#MANDIBULAR L. NODE	(86)	(43)	(45)
LYMPHANGIECTASIS		2 (5%)	1 (2%)
#PANCREATIC L.NODE	(86)	(43)	(45)
LYMPHANGIECTASIS	1 (1%)		
#LUNG	(88)	(48)	(50)
EMBOLUS, FAT		1 (2%)	
#HEART	(88)	(49)	(48)
FIBROSIS, FOCAL	1 (1%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUES)

	CONTROL	LOW DOSE	HIGH DOSE
#HEART/ATRIUM	(88)	(49)	(48)
THROMBOSIS, NOS	1 (1%)		
#LEFT ATRIUM	(88)	(49)	(48)
THROMBOSIS, NOS		1 (2%)	
#MYOCARDIUM	(88)	(49)	(48)
INFLAMMATION, CHRONIC FOCAL	1 (1%)		
FIBROSIS, DIFFUSE	1 (1%)		
DEGENERATION, NOS	11 (13%)	19 (39%)	15 (31%)
*PULMONARY ARTERY	(88)	(50)	(50)
MINERALIZATION			1 (2%)
*MESENTERY	(88)	(50)	(50)
PERIVASCULITIS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(87)	(50)	(48)
ATROPHY, FOCAL		1 (2%)	
METAPLASIA, SQUAMOUS		1 (2%)	
#LIVER	(88)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	2 (2%)	5 (10%)	4 (8%)
INFLAMMATION, CHRONIC	3 (3%)		
INFLAMMATION, CHRONIC FOCAL	19 (22%)		
INFLAMMATION, FOCAL GRANULOMATOU	1 (1%)		2 (4%)
NECROSIS, FOCAL	1 (1%)	1 (2%)	2 (4%)
NECROSIS, CENTRAL	1 (1%)		
BASOPHILIC CYTO CHANGE	62 (70%)	42 (84%)	41 (82%)
FOCAL CELLULAR CHANGE	3 (3%)	4 (8%)	2 (4%)
#PORTAL TRACT	(88)	(50)	(50)
INFLAMMATION, CHRONIC	1 (1%)		
#LIVER/CENTRILOBULAR	(88)	(50)	(50)
NECROSIS, FOCAL	1 (1%)	1 (2%)	1 (2%)
PIGMENTATION, NOS	1 (1%)		
#BILE DUCT	(88)	(50)	(50)
HYPERPLASIA, NOS	6 (7%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, FOCAL	5 (6%)	3 (6%)	1 (2%)
#PANCREATIC ACINUS	(83)	(48)	(50)
DEGENERATION, NOS	1 (1%)		
ATROPHY, NOS	5 (6%)		
ATROPHY, FOCAL	1 (1%)	6 (13%)	8 (16%)
ATROPHY, DIFFUSE	1 (1%)		
#PERIESOPHAGEAL TISSU	(87)	(47)	(49)
INFLAMMATION, CHRONIC	1 (1%)		
#STOMACH	(86)	(50)	(49)
MINERALIZATION		1 (2%)	
ULCER, ACUTE		1 (2%)	
FIBROSIS, DIFFUSE	1 (1%)		
#GASTRIC MUCOSA	(86)	(50)	(49)
NECROSIS, FOCAL	1 (1%)		
#CARDIAC STOMACH	(86)	(50)	(49)
EDEMA, NOS	1 (1%)		
INFLAMMATION, FOCAL	1 (1%)		
INFLAMMATION, VESICULAR	1 (1%)		
ULCER, CHRONIC	1 (1%)		
HYPERPLASIA, EPITHELIAL	1 (1%)	1 (2%)	
#COLON	(72)	(48)	(48)
NEMATODIASIS	6 (8%)		
*RECTUM	(88)	(50)	(50)
NEMATODIASIS	1 (1%)		
*RECTAL MUCOUS MEMBRA	(88)	(50)	(50)
ATROPHY, NOS	1 (1%)		
URINARY SYSTEM			
#KIDNEY	(88)	(50)	(50)
CYST, NOS			1 (2%)
NEPHROPATHY	12 (14%)	9 (18%)	14 (28%)
INFARCT, NOS	1 (1%)		
PIGMENTATION, NOS	3 (3%)	1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	
#KIDNEY/CORTEX	(88)	(50)	(50)
MINERALIZATION		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
PIGMENTATION, NOS	1 (1%)	1 (2%)	
#KIDNEY/TUBULE	(88)	(50)	(50)
PIGMENTATION, NOS	2 (2%)		
REGENERATION, NOS	1 (1%)		
#KIDNEY/PELVIS	(88)	(50)	(50)
MINERALIZATION	2 (2%)	3 (6%)	7 (14%)
#U.BLADDER/SUBMUCOSA	(80)	(45)	(48)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(83)	(46)	(49)
HYPERPLASIA, CHROMOPHOBE-CELL	12 (14%)	7 (15%)	10 (20%)
ANGIECTASIS	2 (2%)	1 (2%)	
#PITUITARY ACIDOPHIL	(83)	(46)	(49)
HYPERPLASIA, NOS	1 (1%)		
#ADRENAL	(86)	(50)	(50)
ABSCESS, CHRONIC	1 (1%)		
LIPOIDOSIS		1 (2%)	1 (2%)
ATROPHY, NOS	1 (1%)		
HYPERTROPHY, FOCAL		1 (2%)	
ANGIECTASIS	1 (1%)		
#ADRENAL CORTEX	(86)	(50)	(50)
CYST, NOS		1 (2%)	
LIPOIDOSIS	15 (17%)	6 (12%)	9 (18%)
FOCAL CELLULAR CHANGE	1 (1%)		
HYPERTROPHY, FOCAL		2 (4%)	2 (4%)
HYPERPLASIA, NOS	5 (6%)		
HYPERPLASIA, FOCAL	7 (8%)	7 (14%)	18 (36%)
ANGIECTASIS	1 (1%)		
#ZONA FASCICULATA	(86)	(50)	(50)
LIPOIDOSIS	1 (1%)		
#ADRENAL MEDULLA	(86)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	1 (1%)	2 (4%)	
HYPERPLASIA, DIFFUSE		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(86)	(49)	(50)
THYROGLOSSAL DUCT CYST			2 (4%)
FOLLICULAR CYST, NOS		1 (2%)	
HYPERPLASIA, C-CELL	16 (19%)	11 (22%)	10 (20%)
HYPERPLASIA, FOLLICULAR-CELL	1 (1%)		
#PANCREATIC ISLETS	(83)	(48)	(50)
HYPERPLASIA, FOCAL		2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(88)	(50)	(50)
DILATATION, NOS	2 (2%)		1 (2%)
DILATATION/DUCTS	3 (3%)		
CYST, NOS	2 (2%)		1 (2%)
CYSTIC DUCTS	2 (2%)		
HYPERPLASIA, NOS	1 (1%)	1 (2%)	1 (2%)
HYPERPLASIA, EPITHELIAL	1 (1%)		
HYPERPLASIA, CYSTIC	19 (22%)		6 (12%)
*MAMMARY ACINUS	(88)	(50)	(50)
DILATATION, NOS	1 (1%)		
CYST, NOS	3 (3%)	5 (10%)	4 (8%)
MULTIPLE CYSTS	1 (1%)		
HYPERPLASIA, NOS	4 (5%)	2 (4%)	1 (2%)
HYPERPLASIA, CYSTIC	2 (2%)	9 (18%)	11 (22%)
*CLITORAL GLAND	(88)	(50)	(50)
CYST, NOS	1 (1%)		
METAPLASIA, SQUAMOUS		1 (2%)	
*VAGINA	(88)	(50)	(50)
POLYP	1 (1%)		
#UTERUS	(87)	(50)	(50)
DILATATION, NOS		5 (10%)	1 (2%)
HYPOPLASIA, NOS			1 (2%)
#CERVIX UTERI	(87)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
#UTERUS/ENDOMETRIUM	(87)	(50)	(50)
NECROSIS, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS	1 (1%)		
HYPERPLASIA, EPITHELIAL		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, CYSTIC			1 (2%)
#ENDOMETRIAL GLAND	(87)	(50)	(50)
CYST, NOS	4 (5%)	8 (16%)	5 (10%)
MULTIPLE CYSTS			9 (18%)
HYPERPLASIA, NOS		1 (2%)	
#OVARY	(86)	(50)	(50)
FOLLICULAR CYST, NOS	3 (3%)	2 (4%)	
HYPOPLASIA, NOS			1 (2%)
#OVARY/RETE OVARII	(86)	(50)	(50)
HYPERPLASIA, NOS	1 (1%)		
#MESOVARIUM	(86)	(50)	(50)
NECROSIS, FAT	1 (1%)		
NERVOUS SYSTEM			
#CEREBRAL VENTRICLE	(88)	(50)	(50)
HYDROCEPHALUS, NOS		1 (2%)	
#BRAIN	(88)	(50)	(50)
HYDROCEPHALUS, NOS	8 (9%)	1 (2%)	3 (6%)
HEMORRHAGE	1 (1%)	1 (2%)	
NECROSIS, FOCAL	1 (1%)		
ATROPHY, PRESSURE	2 (2%)		2 (4%)
#HYPOTHALAMUS	(88)	(50)	(50)
ATROPHY, PRESSURE	6 (7%)	2 (4%)	4 (8%)
#CEREBELLUM	(88)	(50)	(50)
MINERALIZATION	1 (1%)		
SPECIAL SENSE ORGANS			
*EYE	(88)	(50)	(50)
SYNECHIA, ANTERIOR	1 (1%)		
*EYE/RETINA	(88)	(50)	(50)
ATROPHY, NOS	1 (1%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, DIFFUSE	1 (1%)		
*EYE/CRYSTALLINE LENS	(88)	(50)	(50)
DEGENERATION, NOS	2 (2%)		
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(88)	(50)	(50)
DEGENERATION, NOS			1 (2%)
BODY CAVITIES			
*MESENTERY	(88)	(50)	(50)
INFLAMMATION, GRANULOMATOUS			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
NECROSIS, FAT	3 (3%)	1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(88)	(50)	(50)
HEMORRHAGE	1 (1%)		
BACTERIAL SEPTICEMIA		1 (2%)	
BROAD LIGAMENT			
STEATITIS	1		
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY	2		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED DIETS CONTAINING C.I. ACID RED 14

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED DIETS CONTAINING C.I. ACID RED 14

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	48	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
ULCER, NOS	1 (2%)		
ULCER, FOCAL		1 (2%)	
FIBROSIS	1 (2%)	1 (2%)	
*SUBCUT TISSUE	(49)	(50)	(50)
ABSCESS, NOS	1 (2%)		
INFLAMMATION, GRANULOMATOUS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(46)	(49)	(50)
CONGESTION, NOS			1 (2%)
HEMORRHAGE		2 (4%)	1 (2%)
BRONCHOPNEUMONIA, FOCAL	5 (11%)	3 (6%)	
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
INFLAMMATION, INTERSTITIAL	15 (33%)	13 (27%)	10 (20%)
PNEUMONIA INTERSTITIAL CHRONIC	1 (2%)		
GRANULOMA, PYOGENIC		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	22 (48%)	23 (47%)	17 (34%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
HYPERPLASIA, LYMPHOID	1 (2%)		1 (2%)
#BONE MARROW	(46)	(49)	(46)
INFLAMMATION, GRANULOMATOUS			1 (2%)
DEPLETION	4 (9%)		
HYPERPLASIA, GRANULOCYTIC	1 (2%)	3 (6%)	
HYPERPLASIA, RETICULUM CELL		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#SPLEEN	(45)	(49)	(50)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
HEMOSIDEROSIS	1 (2%)		
LYMPHOID DEPLETION	1 (2%)	2 (4%)	1 (2%)
HYPERPLASIA, LYMPHOID	3 (7%)	1 (2%)	2 (4%)
HEMATOPOIESIS		1 (2%)	4 (8%)
#SPLENIC RED PULP	(45)	(49)	(50)
HEMATOPOIESIS	7 (16%)	5 (10%)	4 (8%)
#LYMPH NODE	(36)	(39)	(38)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)
#MANDIBULAR L. NODE	(36)	(39)	(38)
HYPERPLASIA, LYMPHOID		1 (3%)	
#PANCREATIC L.NODE	(36)	(39)	(38)
FIBROSIS	1 (3%)		
#MESENTERIC L. NODE	(36)	(39)	(38)
CONGESTION, NOS	1 (3%)		
ANGIECTASIS			1 (3%)
ERYTHROPHAGOCYTOSIS	2 (6%)		2 (5%)
HEMATOPOIESIS	6 (17%)	4 (10%)	3 (8%)
#LUNG/BRONCHIOLE	(46)	(49)	(50)
HYPERPLASIA, LYMPHOID		1 (2%)	
#LUNG	(46)	(49)	(50)
HYPERPLASIA, LYMPHOID	9 (20%)	11 (22%)	16 (32%)
#SALIVARY GLAND	(45)	(50)	(49)
HYPERPLASIA, LYMPHOID	9 (20%)	6 (12%)	8 (16%)
#LIVER	(48)	(50)	(50)
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS		3 (6%)	
#PANCREAS	(46)	(48)	(48)
HYPERPLASIA, LYMPHOID		1 (2%)	
#KIDNEY	(48)	(50)	(50)
HYPERPLASIA, LYMPHOID	21 (44%)	26 (52%)	20 (40%)
#U.BLADDER/SUBMUCOSA	(45)	(50)	(47)
HYPERPLASIA, LYMPHOID	4 (9%)	1 (2%)	3 (6%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYMIC CORTEX	(27)	(33)	(34)
LYMPHOID DEPLETION		1 (3%)	1 (3%)
CIRCULATORY SYSTEM			
#MESENTERIC L. NODE	(36)	(39)	(38)
LYMPHANGIECTASIS		1 (3%)	
#LUNG	(46)	(49)	(50)
EMBOLISM, NOS		1 (2%)	
PERIVASCULITIS	1 (2%)		
#HEART	(45)	(50)	(50)
PERIVASCULITIS			1 (2%)
#LEFT VENTRICLE	(45)	(50)	(50)
THROMBUS, MURAL			1 (2%)
#MYOCARDIUM	(45)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	1 (2%)
#CARDIAC VALVE	(45)	(50)	(50)
HEMOSIDEROSIS		3 (6%)	
#U.BLADDER/SUBMUCOSA	(45)	(50)	(47)
PERIARTERITIS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(48)	(50)	(50)
CYST, NOS		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU	6 (13%)	3 (6%)	1 (2%)
DEGENERATION, NOS			1 (2%)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL	1 (2%)		3 (6%)
NECROSIS, COAGULATIVE	1 (2%)		
METAMORPHOSIS FATTY			1 (2%)
FOCAL CELLULAR CHANGE	1 (2%)	1 (2%)	1 (2%)
ANGIECTASIS	1 (2%)		
#PORTAL TRACT	(48)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LIVER/CENTRILOBULAR	(48)	(50)	(50)
DEGENERATION, NOS		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
ANGIECTASIS			1 (2%)
#LIVER/PERIPORTAL	(48)	(50)	(50)
NECROSIS, NOS			1 (2%)
#LIVER/KUPFFER CELL	(48)	(50)	(50)
HYPERPLASIA, FOCAL			1 (2%)
*GALLBLADDER	(49)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
#BILE DUCT	(48)	(50)	(50)
MULTIPLE CYSTS		1 (2%)	
#PANCREATIC ACINUS	(46)	(48)	(48)
NECROSIS, FOCAL			1 (2%)
ATROPHY, FOCAL		1 (2%)	
#GASTRIC FUNDUS	(44)	(49)	(46)
GROWTH ARREST		1 (2%)	
#JEJUNUM	(40)	(49)	(45)
HYPERPLASIA, ADENOMATOUS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(50)
MINERALIZATION		2 (4%)	
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	
PYELONEPHRITIS, ACUTE			1 (2%)
SCLEROSIS		1 (2%)	
GLOMERULOSCLEROSIS, NOS	1 (2%)	1 (2%)	1 (2%)
#KIDNEY/CORTEX	(48)	(50)	(50)
MINERALIZATION		1 (2%)	1 (2%)
INFLAMMATION, INTERSTITIAL		2 (4%)	2 (4%)
NEPHROSIS, NOS	1 (2%)		
#KIDNEY/MEDULLA	(48)	(50)	(50)
MINERALIZATION		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#KIDNEY/GLOMERULUS	(48)	(50)	(50)
NEPHROSIS, NOS		1 (2%)	
#KIDNEY/TUBULE	(48)	(50)	(50)
MINERALIZATION			1 (2%)
DILATATION, NOS	1 (2%)		1 (2%)
NEPHROSIS, NOS			1 (2%)
LIPOIDOSIS			1 (2%)
CYTOLOGIC DEGENERATION			1 (2%)
REGENERATION, NOS	27 (56%)	30 (60%)	27 (54%)
#KIDNEY/PELVIS	(48)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
INFLAMMATION, ACUTE FOCAL		1 (2%)	
#URINARY BLADDER	(45)	(50)	(47)
INFLAMMATION, ACUTE NECROTIZING			1 (2%)
#U. BLADDER/MUCOSA	(45)	(50)	(47)
DEGENERATION, NOS		1 (2%)	
#U.BLADDER/SUBMUCOSA	(45)	(50)	(47)
DEGENERATION, HYALINE			1 (2%)
*URETHRA	(49)	(50)	(50)
INFLAMMATION, ACUTE NECROTIZING			1 (2%)
*PROSTATIC URETHRA	(49)	(50)	(50)
HEMORRHAGE		1 (2%)	
INFLAMMATION, ACUTE NECROTIZING			1 (2%)
EROSION		1 (2%)	
NECROSIS, DIFFUSE		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(46)	(47)	(46)
FOCAL CELLULAR CHANGE	1 (2%)	1 (2%)	
HYPERPLASIA, NODULAR			1 (2%)
#THYROID	(44)	(49)	(44)
FOLLICULAR CYST, NOS		1 (2%)	1 (2%)
INFLAMMATION, FOCAL		1 (2%)	
HYPERPLASIA, FOLLICULAR-CELL		2 (4%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYROID FOLLICLE	(44)	(49)	(44)
MULTILOCULAR CYST		1 (2%)	
NUCLEAR CRYSTALLINE AGGREGATE		1 (2%)	
REPRODUCTIVE SYSTEM			
*PENIS	(49)	(50)	(50)
CALCULUS, NOS			1 (2%)
*PREPUCE	(49)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
*PREPUTIAL GLAND	(49)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, GRANULOMATOUS	1 (2%)	1 (2%)	
#PROSTATE	(44)	(49)	(46)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
*SEMINAL VESICLE	(49)	(50)	(50)
PERIVASCULAR CUFFING			1 (2%)
HYPOPLASIA, NOS		1 (2%)	
ATROPHY, NOS		1 (2%)	
#TESTIS	(47)	(50)	(49)
HYPERPLASIA, INTERSTITIAL CELL		1 (2%)	
#TESTIS/TUBULE	(47)	(50)	(49)
MINERALIZATION	1 (2%)	1 (2%)	
*EPIDIDYMIS	(49)	(50)	(50)
INFLAMMATION, MULTIFOCAL	1 (2%)		
INFLAMMATION, ACUTE NECROTIZING		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
INFLAMMATION, GRANULOMATOUS		2 (4%)	
PERIVASCULAR CUFFING			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(44)	(50)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#CEREBRUM	(44)	(50)	(49)
MINERALIZATION	17 (39%)	17 (34%)	20 (41%)
#BRAIN	(44)	(50)	(49)
MINERALIZATION			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(49)	(50)	(50)
INFLAMMATION, MULTIFOCAL		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	
AUTO/NECROPSY/NO HISTO	1		
AUTOLYSIS/NO NECROPSY	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED DIETS CONTAINING C.I. ACID RED 14

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(48)
EDEMA, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(48)
CONGESTION, NOS		2 (4%)	
EDEMA, NOS		2 (4%)	
BRONCHOPNEUMONIA, FOCAL	5 (10%)	3 (6%)	2 (4%)
INFLAMMATION, INTERSTITIAL	22 (44%)	18 (36%)	15 (31%)
PNEUMONIA INTERSTITIAL CHRONIC	1 (2%)		1 (2%)
INFLAMMATION WITH FIBROSIS	1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	32 (64%)	26 (52%)	22 (46%)
METAPLASIA, OSSEOUS			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID	11 (22%)	11 (22%)	10 (21%)
#BONE MARROW	(47)	(48)	(46)
FIBROUS OSTEODYSTROPHY		1 (2%)	
ANGIECTASIS	1 (2%)		
PLASMACYTOSIS		1 (2%)	
HYPERPLASIA, GRANULOCYTIC	1 (2%)	2 (4%)	1 (2%)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HYPOPLASIA, HEMATOPOIETIC	3 (6%)		
HYPOPLASIA, ERYTHROID		1 (2%)	
#SPLEEN	(48)	(50)	(48)
HEMORRHAGE	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
LYMPHOID DEPLETION		3 (6%)	3 (6%)
ANGIECTASIS	1 (2%)		
LEUKEMOID REACTION		1 (2%)	
HYPERPLASIA, LYMPHOID		8 (16%)	10 (21%)
HEMATOPOIESIS	1 (2%)	1 (2%)	
#SPLENIC RED PULP	(48)	(50)	(48)
HEMOSIDEROSIS	1 (2%)		
LYMPHOID DEPLETION		1 (2%)	
HEMATOPOIESIS	10 (21%)	10 (20%)	4 (8%)
#LYMPH NODE	(41)	(38)	(37)
HYPERPLASIA, LYMPHOID		1 (3%)	
#LUNG	(50)	(50)	(48)
LEUKEMOID REACTION			1 (2%)
HYPERPLASIA, LYMPHOID	12 (24%)	7 (14%)	13 (27%)
#SALIVARY GLAND	(48)	(48)	(46)
HYPERPLASIA, LYMPHOID	4 (8%)	8 (17%)	7 (15%)
#LIVER	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID	4 (8%)	1 (2%)	
HEMATOPOIESIS	2 (4%)	16 (32%)	10 (21%)
#LIVER/CENTRILOBULAR	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)		
#PANCREAS	(48)	(49)	(46)
HYPERPLASIA, LYMPHOID	6 (13%)	2 (4%)	2 (4%)
#KIDNEY	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID	16 (32%)	25 (50%)	20 (42%)
#URINARY BLADDER	(47)	(48)	(47)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	1 (2%)
#U.BLADDER/SUBMUCOSA	(47)	(48)	(47)
HYPERPLASIA, LYMPHOID	8 (17%)	19 (40%)	20 (43%)
#ADRENAL	(46)	(48)	(48)
HEMATOPOIESIS	1 (2%)		
#THYMUS	(35)	(39)	(42)
INVOLUTION, NOS	1 (3%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
METAPLASIA, OSSEOUS	1 (3%)		
#THYMIC CORTEX	(35)	(39)	(42)
LYMPHOID DEPLETION		3 (8%)	2 (5%)
CIRCULATORY SYSTEM			
#LUNG	(50)	(50)	(48)
PERIVASCULITIS			2 (4%)
#HEART	(50)	(50)	(48)
PERIARTERITIS		1 (2%)	2 (4%)
#MYOCARDIUM	(50)	(50)	(48)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	2 (4%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
DEGENERATION, NOS	1 (2%)		
#CARDIAC VALVE	(50)	(50)	(48)
HEMOSIDEROSIS	4 (8%)	5 (10%)	5 (10%)
#U.BLADDER/SUBMUCOSA	(47)	(48)	(47)
PERIARTERITIS			2 (4%)
#OVARY	(45)	(47)	(44)
THROMBOSIS, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(48)	(48)	(46)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
#LIVER	(50)	(50)	(48)
INFLAMMATION, ACUTE/CHRONIC	2 (4%)	2 (4%)	5 (10%)
INFLAMMATION, FOCAL GRANULOMATOU	7 (14%)	15 (30%)	14 (29%)
DEGENERATION, NOS			1 (2%)
FOCAL CELLULAR CHANGE	1 (2%)		
#LIVER/CENTRILOBULAR	(50)	(50)	(48)
NECROSIS, FOCAL		2 (4%)	
ANGIECTASIS	1 (2%)		
#LIVER/KUPFFER CELL	(50)	(50)	(48)
HYPERPLASIA, FOCAL		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, DIFFUSE		1 (2%)	1 (2%)
*GALLBLADDER	(50)	(50)	(48)
CYST, NOS	1 (2%)		
INFLAMMATION, FOCAL	1 (2%)		
#BILE DUCT	(50)	(50)	(48)
DILATATION, NOS	1 (2%)		
CYST, NOS	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
#PANCREAS	(48)	(49)	(46)
DILATATION/DUCTS	3 (6%)	1 (2%)	
CYSTIC DUCTS			1 (2%)
INFLAMMATION, MULTIFOCAL			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	2 (4%)
#PANCREATIC DUCT	(48)	(49)	(46)
MULTIPLE CYSTS		1 (2%)	
#PANCREATIC ACINUS	(48)	(49)	(46)
ATROPHY, NOS	1 (2%)		
ATROPHY, FOCAL			2 (4%)
ATROPHY, DIFFUSE		1 (2%)	1 (2%)
#PERIPANCREATIC TISSU	(48)	(49)	(46)
NECROSIS, FAT			1 (2%)
#STOMACH	(50)	(48)	(48)
INFLAMMATION, MULTIFOCAL		1 (2%)	
#JEJUNUM	(44)	(47)	(45)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(48)
HYDRONEPHROSIS			1 (2%)
INFLAMMATION, INTERSTITIAL	1 (2%)		1 (2%)
GLOMERULONEPHRITIS, CHRONIC		1 (2%)	
GLOMERULOSCLEROSIS, NOS	3 (6%)	3 (6%)	2 (4%)
#KIDNEY/CORTEX	(50)	(50)	(48)
MINERALIZATION	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CYST, NOS			1 (2%)
INFLAMMATION, INTERSTITIAL		1 (2%)	
NEPHROSIS, NOS		1 (2%)	
#KIDNEY/TUBULE	(50)	(50)	(48)
MINERALIZATION			1 (2%)
DILATATION, NOS	1 (2%)		
HEMOSIDEROSIS	2 (4%)		
REGENERATION, NOS	12 (24%)	7 (14%)	7 (15%)
#URINARY BLADDER	(47)	(48)	(47)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
INFLAMMATION, MULTIFOCAL			1 (2%)
#U.BLADDER/SUBMUCOSA	(47)	(48)	(47)
EDEMA, NOS			3 (6%)
INFLAMMATION, MULTIFOCAL		2 (4%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(36)	(33)	(41)
CYTOPLASMIC VACUOLIZATION			1 (2%)
FOCAL CELLULAR CHANGE			1 (2%)
HYPERPLASIA, CHROMOPHOBE-CELL	1 (3%)	1 (3%)	1 (2%)
ANGIECTASIS	1 (3%)		
#PITUITARY ACIDOPHIL	(36)	(33)	(41)
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL	(46)	(48)	(48)
HEMORRHAGE	1 (2%)		
NECROSIS, NOS	1 (2%)		
ANGIECTASIS		1 (2%)	
#ADRENAL CORTEX	(46)	(48)	(48)
CYST, NOS		1 (2%)	1 (2%)
DEGENERATION, NOS	1 (2%)		
HYPERPLASTIC NODULE		1 (2%)	
#ADRENAL MEDULLA	(46)	(48)	(48)
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(48)	(49)	(48)
FOLLICULAR CYST, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, FOLLICULAR-CELL	1 (2%)		1 (2%)
#PARATHYROID	(22)	(20)	(26)
CYST, NOS			1 (4%)
ATROPHY, NOS			1 (4%)
HYPERPLASIA, NOS		1 (5%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(48)
DILATATION/DUCTS		2 (4%)	2 (4%)
*MAMMARY ACINUS	(50)	(50)	(48)
HYPERPLASIA, FOCAL	1 (2%)		
HYPERPLASIA, DIFFUSE		1 (2%)	
#UTERUS	(49)	(49)	(48)
DILATATION, NOS			1 (2%)
#UTERUS/ENDOMETRIUM	(49)	(49)	(48)
HYPERPLASIA, CYSTIC			1 (2%)
#ENDOMETRIAL GLAND	(49)	(49)	(48)
HYPERPLASIA, CYSTIC	34 (69%)	39 (80%)	37 (77%)
#OVARY	(45)	(47)	(44)
CYST, NOS	7 (16%)	7 (15%)	7 (16%)
MULTILOCULAR CYST	1 (2%)	1 (2%)	
PAROVARIAN CYST			1 (2%)
HEMORRHAGIC CYST	2 (4%)		1 (2%)
INFLAMMATION, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(49)	(50)	(48)
PERIVASCULAR CUFFING	3 (6%)		3 (6%)
#CEREBRUM	(49)	(50)	(48)
MINERALIZATION	17 (35%)	12 (24%)	20 (42%)
#BRAIN	(49)	(50)	(48)
MINERALIZATION		1 (2%)	
PERIVASCULAR CUFFING	2 (4%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*SPINAL NERVE	(50)	(50)	(48)
INFLAMMATION, ACUTE FOCAL			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*FEMUR	(50)	(50)	(48)
EXOSTOSIS			1 (2%)
*SKELETAL MUSCLE	(50)	(50)	(48)
INFLAMMATION, FOCAL		1 (2%)	
BODY CAVITIES			
*ABDOMINAL WALL	(50)	(50)	(48)
NECROSIS, NOS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
AUTOLYSIS/NO NECROPSY			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX E

ANALYSIS OF C.I. ACID RED 14
(LOT NO. A77716)
MIDWEST RESEARCH INSTITUTE

Appendix E

Analysis of C.I. Acid Red 14
(Lot No. A77716)
Midwest Research Institute

A. ELEMENTAL ANALYSIS

Element:	C	H	N	S	Na	Cl
Theory:	47.81	2.41	5.58	12.76	9.15	--
Theory(a):	34.14	2.55	3.98	9.11	11.13	7.10
Determined:	34.16	2.64	3.65	10.42	14.2±0.1(δ)	7.1±0.3(δ)
	34.38	2.69	3.69	10.48		

(a) Theoretical composition based on 71.4% C.I. Acid Red 14 as determined by titration, 7.4% water from the Karl Fisher analysis, and 11.7% sodium chloride based on the determined chloride content.

B. WATER ANALYSIS

(Karl Fisher)
7.39±0.09 (δ)%

C. TITRATION WITH TITANOUS CHLORIDE

71.4±0.(δ)2%. Modification of the method (Horowitz, 1975): sample 0.02 (δ)% weighed directly into titration vessel.

D. MELTING POINT

Determined	Literature Values
290°-320°C dec. (visual capillary)	No literature value found

E. THIN-LAYER CHROMATOGRAPHY

Plates: Silica gel 60 F254
Amount Spotted: 100 and 300 μg
Ref. Standard: Methyl red
Visualization: Visible and ultraviolet light (366 and 254 nm)

System 1: 95% ethanol:concentrated ammonium hydroxide (90:10)
R_f: 0.91 (trace); 0.83 (slight trace); 0.72 (trace); 0.63 (major)
R_{st}: 1.10; 1.00; 0.88; 0.76

System 2: n-Butanol:methyl ethyl ketone:concentrated aqueous ammonium hydroxide:water (50:30:10:10)
R_f: 0.66 (trace); 0.56 (trace); 0.32 (trace); 0.15 (slight trace); 0.12 (trace); 0.09 (major)
R_{st}: 2.10; 1.85; 1.00; 0.46; 0.38; 0.28

F. HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Instrument: Waters ALC 202 with Model 660 solvent programmer.
Detector: Ultraviolet 254 nm
Column: C_{18} μ-Bondapak, 300 x 4 mm I.D.
Solvent: 63% B
A - 0.005M tetrabutylammonium hydroxide and 1% acetic acid in water
B - 0.005M tetrabutylammonium hydroxide and 1% acetic acid in methanol

Flow rate: 1.5 ml/min
Results: Major peak and three impurities

Peak	Retention Time (min)	Retention Time (Relative to C.I. Acid Red 14)	Area (Relative to C.I. Acid Red 14)
Minor	2.8	0.31	0.52
Minor	7.5	0.83	0.78
Major	9.0	1.00	100.00
Minor	13.0	1.44	0.52

G. SPECTRAL DATA

(1) Infrared:

Instrument: Beckman IR-12
Cell: 1.5% potassium bromide pellet
Results: See Figure 5

No literature spectrum found. Spectrum consistent with structure.

(2) Ultraviolet/Visible

Instrument: Cary 118

λ max (nm)	$\epsilon \times 10^{-3}$
218	43.9±0.4 (δ)
235 (shoulder)	17.4±0.2 (δ)
281	10.5±0.1 (δ)
292	11.1±0.1 (δ)
322	12.2±0.1 (δ)
334 (shoulder)	11.5±0.1 (δ)
405 (shoulder)	4.36±0.02 (δ)
518	19.2±0.2 (δ)
Solvent: Water	

λ max (nm)	$\epsilon \times 10^{-3}$
220	-
290	-
320	-
515	26.1
Solvent:	0.02 M ammonium acetate (Pellerin et al., 1963)
510	17.6
Solvent:	7.4 buffer (Jones and Thomas, 1968)

(3) Nuclear magnetic resonance

Instrument: Varian HA-100
Solvent: Dimethylsulfoxide-d_6
D_2O (1:1) with internal sodium 3-trimethylsilylpropionate-2,2,3,3-d_4
Assignments: (see Figure 6)

No literature reference found

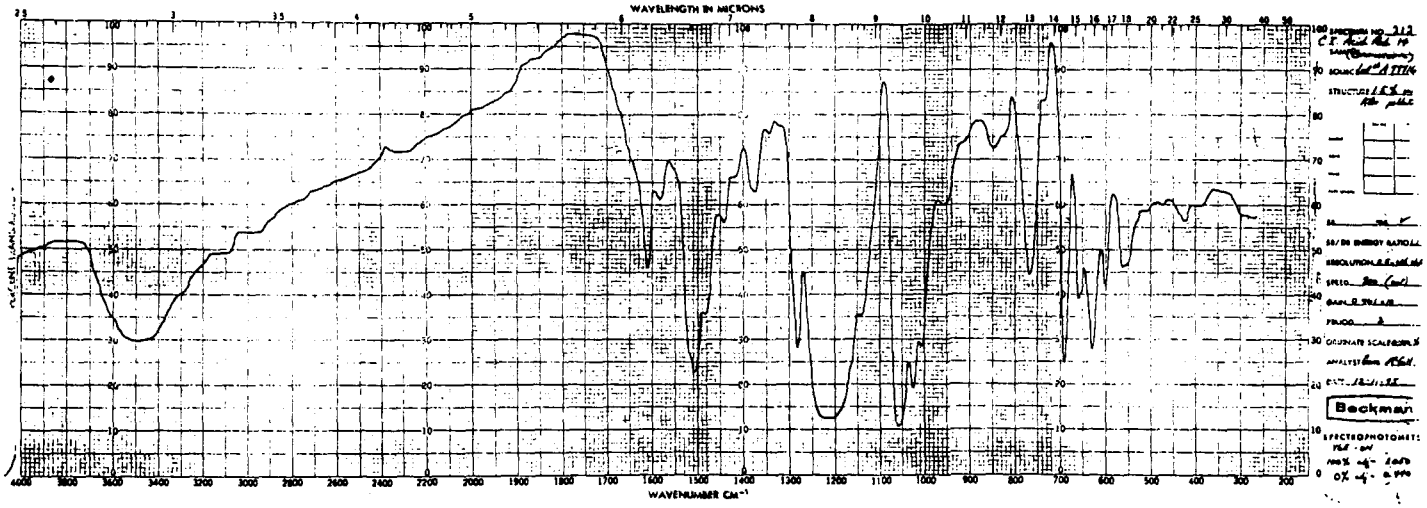

Figure 5. Infrared Absorption Spectrum of C.I. Acid Red 14 (Lot No. A77716)

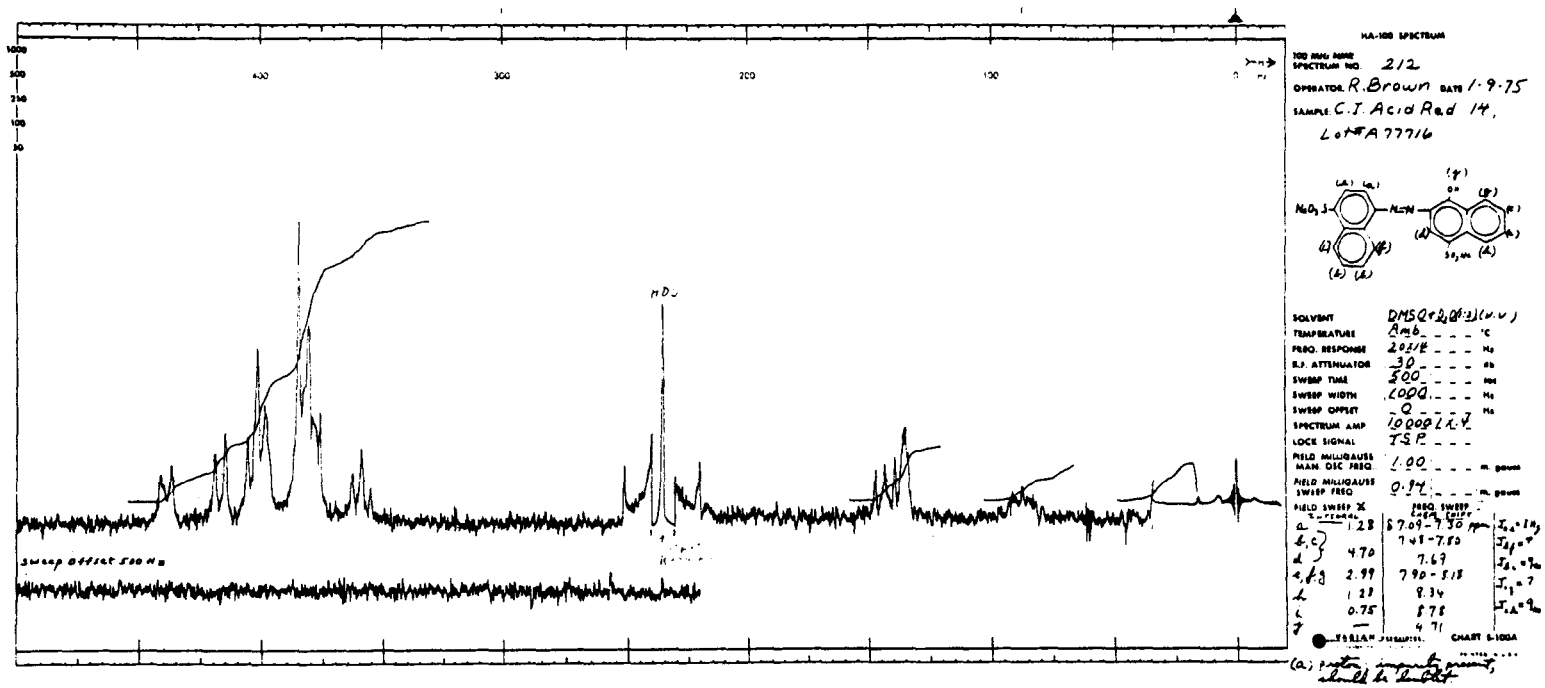

Figure 6. Nuclear Magnetic Resonance Spectrum of C.I. Acid Red 14 (Lot No. A77716)

(a) δ = 7.14 ppm
(b)(c) δ = 7.48-7.80 ppm
(d) δ = 7.69 ppm
(e,f,g) δ = 7.90-8.18 ppm
(h) δ = 8.34 ppm
(i) δ = 8.78
(j) δ = 4.71 ppm
(k) δ = 7.26 ppm (impurity)

J_{ae} = 8 Hz
J_{bi} = 9 Hz
J_{ch} = 9 Hz

Integration ratios:

(a) = 0.80
(b,c,d) = 4.70
(e,f,g) = 2.99
(h) = 1.28
(i) = 0.75
(j) = under solvent
(k) = 0.48 (impurity)

APPENDIX F

ANALYSIS OF C.I. ACID RED 14
(LOT NO. A83650)
MIDWEST RESEARCH INSTITUTE

Appendix F
Analysis of C.I. Acid Red 14
(Lot No. A83650)
Midwest Research Institute

A. ELEMENTAL ANALYSIS

Element:	C	H	N	S	Na	Cl
Theory:	47.81	2.41	5.58	12.76	9.15	--
Theory(a):	32.17	2.45	3.75	11.35	13.20(b)	4.76
Determined:	33.00	2.50	3.97	11.29	14.63	4.76±0.17(δ)
	32.91	2.45	3.84	11.38	14.39	

(a) Theory based on 67.3% compound (by titration), 7.48% H_2O (by Karl Fisher), 7.85% NaCl (from determined chloride content), and 12.21% Na_2SO_4 (based on calculations from sulfur and sodium content).

(b) It is possible that the 1.3% excess of sodium is present as carbonates.

B. WATER ANALYSIS

(Karl Fisher)
7.48±0.12 (δ)%

C. TITRATION WITH TITANOUS CHLORIDE

67.3%±0.5(δ)% Modification of the method (Horowitz, 1975): sample weighed directly into titration vessel.

D. THIN-LAYER CHROMATOGRAPHY

Plates: Silica gel G-25
UV_{254}

Ref. Standard: Methyl red
(10.0μg/μl methanol)

Amount Spotted: 100 and 300μg (10.0μg/μl methanol)

Visualization: Visible and ultraviolet light (366 nm and 254 nm)

System 1: Ethanol:concentrated ammonium hydroxide (90:10)

System 2: n-Butanol:methyl ethyl ketone:concentrated ammonium hydroxide:water (50:30:10:10)

R_f: 0.49 (major), 0.64 (slight trace, 366 nm only)

R_f: 0.15 (major), 0.37 (trace, 366 nm only)

R_{st}: 0.71, 0.92

R_{st}: 0.42, 1.01

E. HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Instrument: Waters ALC 202 with Model 660 solvent programmer
Detector: Ultraviolet, 254 nm
Column: $C_{18}\mu$ Bondapak
Solvent: 55A:45B, isocratic

A. 0.005 M tetrabutyl ammonium hydroxide in water with phosphoric acid to adjust pH to 7.4
B. 0.005 M tetrabutyl ammonium hydroxide in methanol (Fischer HPLC) with equivalent amount of phosphoric acid added as for Solvent A

Peak	Retention Time (min)	Retention Time (Relative to C.I. Acid Red 14)	Area (Relative to C.I. Acid Red 14)
1	6.6	0.79	0.57
2	8.4	1.0	100

F. SPECTRAL DATA

(1) Infrared:

Instrument: Beckman IR-12
Cell: 1.2% potassium bromide pellet

Results: See Figure 7

No literature spectrum found. Spectrum consistent with structure and with spectrum obtained previously at MRI (Lot No. A77716).

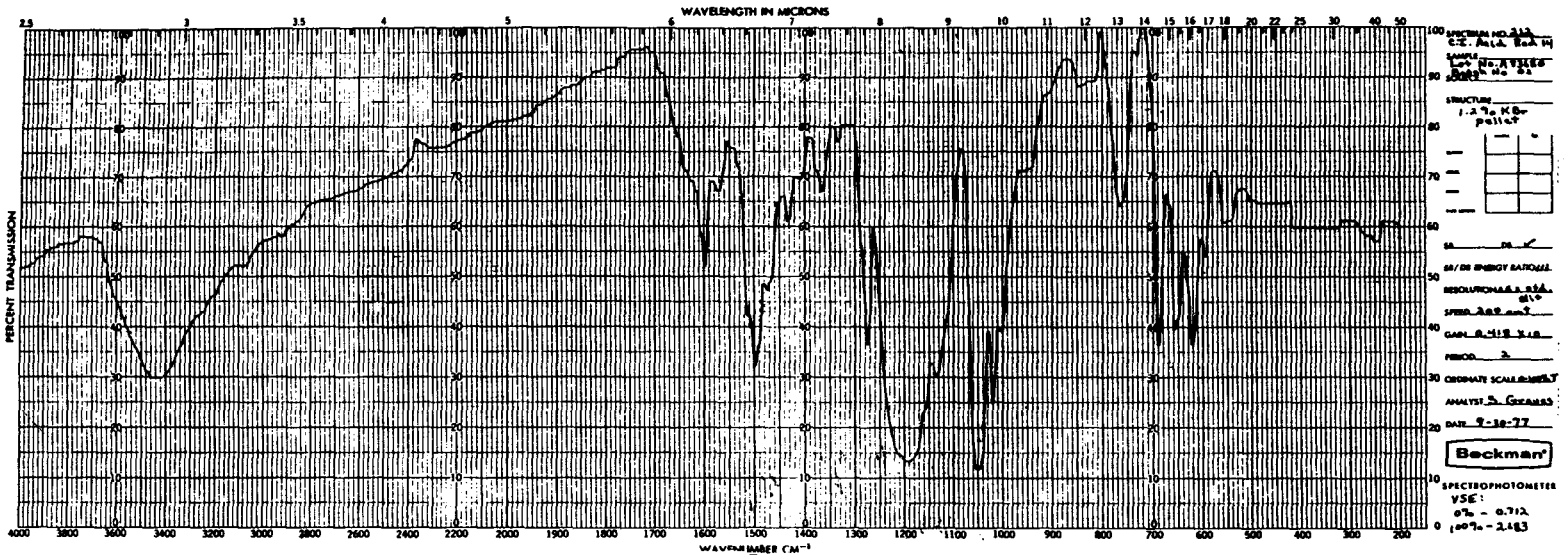

Figure 7. Infrared Absorption Spectrum of C.I. Acid Red 14 (Lot No. A83650)

(2) Ultraviolet/Visible

Instrument: Cary 118

Determined			Literature Values	
λ max (nm)		ϵ x 10^{-4}	λ max (nm)	ϵ x 10^{-4}
217		4.25±0.06(δ)	220	-
233	(shoulder)	1.67±0.02(δ)	290	-
281		0.94±0.01(δ)	320	-
292		0.98±0.01(δ)	515	2.61
320		1.09±0.01(δ)	Solvent: 0.02 M ammonium acetate (Pellerin et al., 1963)	
334	(shoulder)	1.05±0.01(δ)		
400	(shoulder)	0.385±0.002(δ)		
517		1.77±0.09(δ)		
556	(shoulder)	1.25±0.02(δ)	510	1.96
Solvent: 0.02M ammonium acetate			Solvent: 7.4 buffer (Jones and Thomas, 1968)	

(a) The absorbance of this lot at 517 nm is 67.8% of that reported in the literature.

(3) Nuclear Magnetic Resonance

Instrument: Varian EM-360A

Solvent: DMSO:D_2O (1:2, v:v) with internal sodium 3-trimethylsilyl-propionate-2,2,3,3-d_4)

No literature reference found. Consistent with structure and previous spectrum obtained at MRI (Lot No. A77716).

Assignments: (see Figure 8)

(a) δ 7.28 ppm
(b,c) δ 7.58-7.86
(d) δ 7.82 ppm
(e,f,g) δ 7.96-8.16 ppm
(h) δ 8.25 ppm
(i) δ 8.72 ppm
(j) δ 4.72 ppm
(k) δ 1.04 ppm (impurity)
(l) δ 3.38 ppm (impurity)

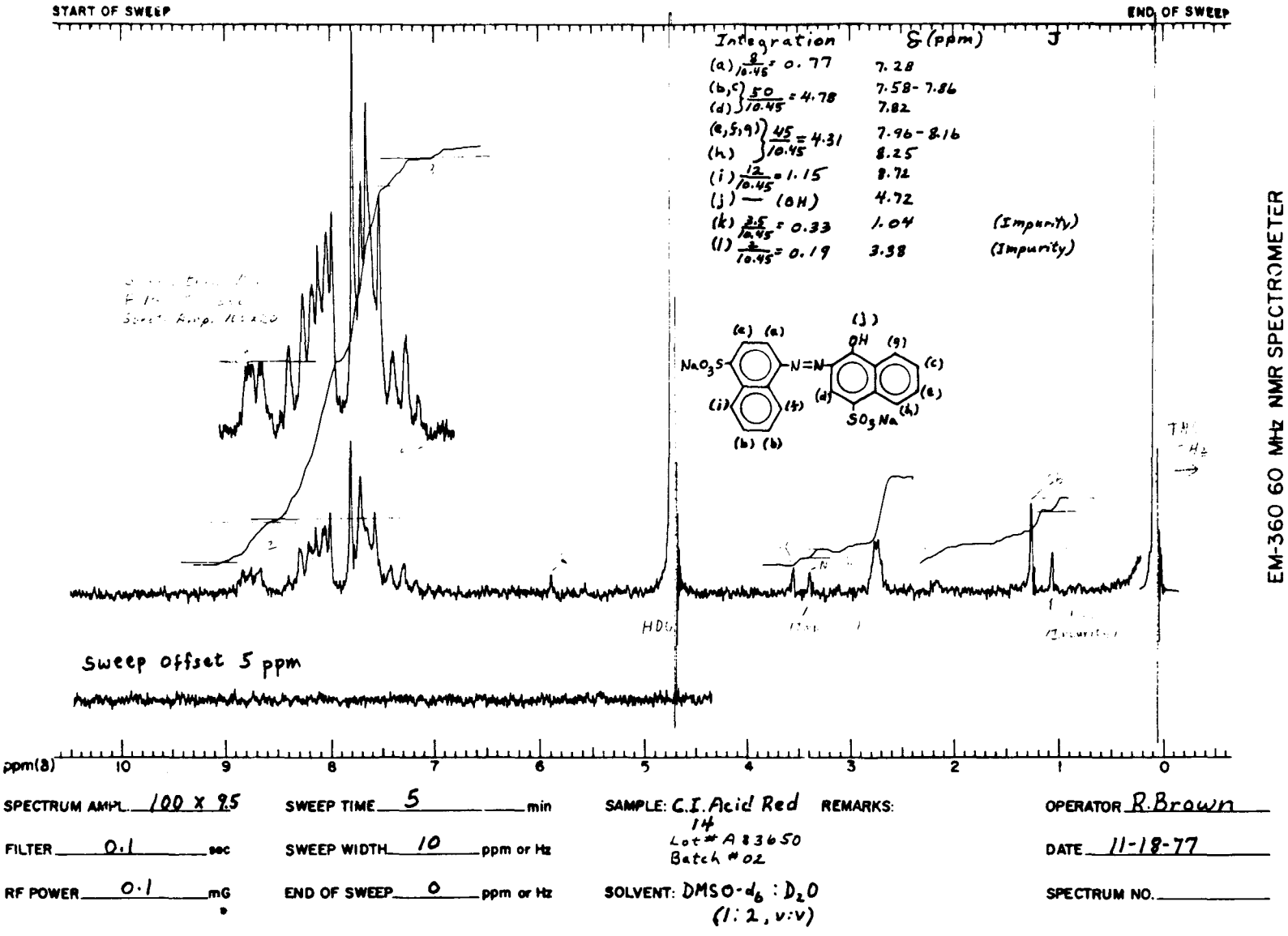

Figure 8. Nuclear Magnetic Resonance Spectrum of C.I. Acid Red 14 (Lot No. A83650)

APPENDIX G

Theoretical Calculation of Batch Composition

APPENDIX G

Theoretical Calculation of Batch Composition

Based on the amounts of pure dye, water, and elemental composition that were experimentally determined (see Appendixes E and F), the composition of each batch was determined to be:

	Lot No. A77716	Lot No. A83650
C.I. Acid Red 14	71.4%	67.30%
H_2O	7.39	7.48
NaCl	11.70	7.85
Na_2SO_4	5.79	12.20
$NaHCO_3$	3.72	5.16

The values for the elemental composition of each, based on these components, compare favorably with actual determined values as shown below.

Lot No. A77716

Element:	C	H	N	S	Na	Cl
Theory:	34.67	2.59	3.98	10.42	14.02	7.10
Determined (a):	34.27	2.66	3.67	10.45	14.20	7.10

Lot No. A83650

Element:	C	H	N	S	Na	Cl
Theory:	32.92	2.52	3.75	11.34	14.61	4.76
Determined (a):	32.95	2.48	3.90	11.34	14.66	4.76

(a) Average values from results at Midwest Research Institute (Appendixes E and F).

APPENDIX H

Analyses of Formulated Diets for Concentrations of C.I. Acid Red 14

APPENDIX H

Analyses of Formulated Diets for Concentrations of C.I. Acid Red 14

A 100-milligram sample of the dye-feed mixture was mixed with 40 ml of 0.025N NaOH and vortexed for 30 seconds. The supension was centrifuged for 10 minutes at 10,000 rpm in a Sorvall RC-2B centrifuge at 4° C. An appropriate volume of the supernatant was removed and diluted with distilled water to achieve a final concentration in the linear portion of the standard curve. Internal standards were prepared using plain feed assayed in the same manner. All samples and standards were run in triplicate. The absorbance was determined at 519 nm in a Gilford 2400-S spectrophotometer. The spectrophotometer was blanked with a 100-mg feed sample treated in the same manner as the samples. The standard curve developed with feed-dye standards (triplicate) automatically incorporated a correction for recovery. The concentration of dye in a feed sample could be read directly from the curve without any further adjustment for recovery.

Theoretical Dietary Level (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
3,000	10	2,996	3.9	2,880 - 3,140
6,000	21	5,954	3.4	5,670 - 6,380
12,500	20	12,411	4.6	11,140 - 13,300
25,000	11	24,551	3.7	23,700 - 26,400

APPENDIX I

Analysis of Formulated Diets for Stability of C.I. Acid Red 14

APPENDIX I

Analysis of Formulated Diets for Stability
of C.I. Acid Red 14

HEAT STABILITY

1. Mixing and storage: C.I. Acid Red 14 (3.057 g) and Wayne Lab-Blox® Rodent Feed (24.577 g) were mixed in a mortar. Samples of the mixture were removed and stored for 2 weeks at -20°, 5°, 25°, and 45°C, respectively. These samples were then analyzed by visible spectrophotometry, as described below.

2. Extraction: One-gram samples of each of the above mixtures were triturated twice with 50-ml portions of Fisher pH 10.4 buffer solution (THAM®) using a Polytron® high-speed blender. Two-milliliter aliquots of this solution (made up exactly to 100 ml) were further diluted to 100 ml with buffer in a volumetric flask and analyzed spectrophotometrically in the visible region at 519 nm.

3. Analytical Results:

Instrument: Cary 118

Sample (°C)	Average Percent Compound (a)
-20	10.3±0.4
5	9.6±0.4
25	9.6±0.4
45	10.1±0.4

(a) Corrected for a spiked recovery value of 96.6%.

4. Conclusion: There is no significant difference between the samples stored at the various temperatures. C.I. Acid Red 14 mixed with feed is stable for 2 weeks at temperatures up to 45°C.

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