NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 285

TOXICOLOGY AND CARCINOGENESIS STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

(PARAROSANILINE)

(CAS NO. 569-61-9)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

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

NTP TECHNICAL REPORT

ON THE

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

January 1986

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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NOTE TO THE READER

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for testing in the NTP Carcinogenesis 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 has the potential for 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.

Five categories of interpretative conclusions were adopted for use in June 1983 in the Technical Reports series to specifically emphasize consistency and the concept of actual evidence of carcinogenicity. For each definitive study result (male rats, female rats, male mice, female mice), one of the following quintet will be selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of malignant neoplasms, studies that exhibit a substantially increased incidence of benign neoplasms, or studies that exhibit an increased incidence of a combination of malignant and benign neoplasms where each increases with dose.
- **Some Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of benign neoplasms, studies that exhibit marginal increases in neoplasms of several organs/tissues, or studies that exhibit a slight increase in uncommon malignant or benign neoplasms.
- **Equivocal Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related marginal increase of neoplasms.
- **No Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate Study of Carcinogenicity** demonstrates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as valid for showing either the presence or absence of a carcinogenic effect.

Additionally, the following concepts (as patterned from the International Agency for Research on Cancer Monographs) have been adopted by the NTP to give further clarification of these issues:

> The term *chemical carcinogenesis* generally means the induction by chemicals of neoplasms not usually observed, the earlier induction by chemicals of neoplasms that are commonly observed, or the induction by chemicals of more neoplasms than are generally found. Different mechanisms may be involved in these situations. Etymologically, the term *carcinogenesis* means induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign neoplasms. In the Technical Reports, the words *tumor* and *neoplasm* are used interchangeably.

This study was initiated by the National Cancer Institute's Carcinogenesis Bioassay Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program. The studies described in this Technical Report have been conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. All NTP toxicology and carcinogenesis studies are subjected to a data audit before being presented for peer review.

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to identify any mistakes 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. Comments and questions about the National Toxicology Program Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3780).

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge (and while supplies last) from the NTP Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709.

CONTENTS

TABLES

TABLES (Continued)

FIGURES

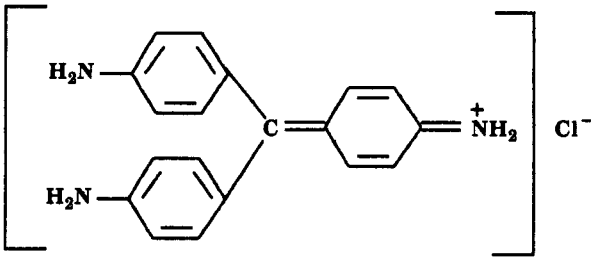
APPENDIXES

APPENDIXES (Continued)

APPENDIXES (Continued)

APPENDIXES (Continued)

APPENDIXES (Continued)



C.I. BASIC RED 9 MONOHYDROCHLORIDE

$C_{19}H_{17}N_3 \cdot HCl$

Molecular Weight: 323.8 **CAS No. 569-61-9**

**(Pararosaniline;
Benzenamine 4-((4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl)- monohydrochloride;
Paramagenta)**

ABSTRACT

C.I. Basic Red 9 monohydrochloride is a triphenylmethane dye used for coloring textiles, leather, and paper and as a biologic stain. Toxicology and carcinogenesis studies were conducted by administering the test chemical in feed to groups of 50 male and 50 female F344/N rats and B6C3F$_1$ mice for 103 weeks at concentrations of 0, 1,000, or 2,000 ppm for male rats and 0, 500, or 1,000 ppm for female rats and mice of each sex. The average daily doses of C.I. Basic Red 9 monohydrochloride were estimated to be 49 and 103 mg/kg for male rats, 28 and 59 mg/kg for female rats, 196 and 379 mg/kg for male mice, and 149 and 407 mg/kg for female mice. Two lots of the test chemical were used in the 2-year studies with purities of 93% (water content approximately 9%) and 99%.

In rats, the thyroid gland and pituitary gland were identified as target sites in the 13-week studies. Therefore, 10 additional rats of each sex were added to the control and high dose groups in the 2-year studies to examine the effects on these organs after 1 year of exposure.

In the 1-year studies in rats, final mean body weights were slightly decreased in both sexes. The thyroid gland weight to body weight ratio of dosed males was 1.7 times that of the controls, and the concentration of serum thyroxin in male and female rats was significantly lower than that of the controls at week 52. Compound-related histopathologic effects included thyroid gland cysts in both sexes (1/10; 1/10) and thyroid gland follicular cell hyperplasia (1/10), adenomas (1/10), and carcinomas (1/10) and fatty metamorphosis of the liver (4/10, two of these with focal necrosis) in males; no effect was seen in the controls.

The doses selected for the 2-year studies were based on the results of the 13-week studies. The absence of toxicologic signs, histopathologic changes, significant body weight depressions, or mortality after 13 weeks of exposure to C.I. Basic Red 9 monohydrochloride suggested that these concentrations would not shorten survival. However, throughout the 2-year studies, mean body weights of high dose rats and dosed mice were lower than those of the controls, and significantly reduced survival relative to controls was observed for high dose rats of each sex ($P < 0.001$), low dose male mice ($P < 0.03$), and low dose and high dose female mice ($P < 0.001$).

In the 2-year studies, several types of neoplastic lesions occurred with significantly increased incidences in dosed animals (see the following tables). High dose male rats had increased incidences of squamous cell carcinomas, trichoepitheliomas, and sebaceous adenomas of the skin. Greater incidences of follicular cell carcinomas and of follicular cell adenomas were found in the thyroid glands of high dose male rats than in controls, whereas in high dose female rats, the combined incidence of follicular cell adenomas or carcinomas was greater than that in controls. Dosed rats of each sex had increased incidences of subcutaneous fibromas, and high dose rats had increased incidences of Zymbal gland carcinomas. Hepatocellular carcinomas were the compound-related neoplasms common to both species; the incidences were increased in high dose male rats and in dosed mice of each sex. Dosed female mice had an increased incidence of pheochromocytomas or malignant pheochromocytomas. In addition, marginally increased incidences of mammary gland tumors (23/50; 32/50; 32/50) in female rats, and malignant lymphomas (17/50; 24/50; 25/50) in female mice were observed.

SUMMARY OF INCIDENCES OF PRIMARY NEOPLASMS IN RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Site/Lesion	Control	Low Dose	High Dose
MALE RATS			
Skin			
Squamous cell carcinoma	0/50	1/50	10/50
Trichoepithelioma	0/50	0/50	7/50
Sebaceous adenoma	0/50	0/50	5/50
Thyroid gland			
Follicular cell adenoma	0/49	0/46	9/44
Follicular cell carcinoma	0/49	5/46	18/44
Subcutaneous tissue			
Fibroma	2/50	20/50	16/50
Zymbal gland			
Carcinoma	1/50	1/50	13/50
Liver			
Neoplastic nodule	5/50	14/50	6/50
Hepatocellular carcinoma	0/50	2/50	8/50
FEMALE RATS			
Thyroid gland			
Follicular cell adenoma or carcinoma	0/47	2/48	6/50
Subcutaneous tissue			
Fibroma	0/50	15/50	10/50
Zymbal gland			
Carcinoma	0/50	2/50	7/50
MALE MICE			
Liver			
Hepatocellular carcinoma	10/50	20/50	27/50
FEMALE MICE			
Liver			
Hepatocellular adenoma	2/49	18/50	4/49
Hepatocellular carcinoma	3/49	19/50	37/49
Adrenal gland			
Pheochromocytoma or malignant pheochromocytoma	1/48	8/47	8/45

SITES OF NEOPLASTIC LESIONS IN RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Site of Neoplastic Lesion	Rats		Mice	
	Male	Female	Male	Female
Skin	+	–	–	–
Subcutaneous tissue	+	+	–	–
Thyroid gland	+	+	–	–
Zymbal gland	+	+	–	–
Liver	+			
Adrenal gland	–	–		
Mammary gland	–	±	–	–
Hematopoietic system	–	–	–	±

+ = Clear evidence of carcinogenicity
± = May have been related to compound exposure
– = No significant increase relative to controls

C.I. Basic Red 9 monohydrochloride was mutagenic in strains TA98 and TA100 of *Salmonella typhimurium* by the preincubation protocol with or without metabolic activation. It was not mutagenic in strains TA1535 and TA1537 in this system with or without metabolic activation. It was mutagenic in the L5178Y/TK$^{+/-}$ mouse lymphoma assay with or without metabolic activation. C.I. Basic Red 9 monohydrochloride did not induce chromosomal aberrations in Chinese hamster ovary cells; it did induce sister-chromatid exchanges in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver S9. C.I. Basic Red 9 monohydrochloride also induced unscheduled DNA synthesis in F344 male rat hepatocytes in vitro.

An audit of the experimental data was conducted for these 2-year studies of C.I. Basic Red 9 monohydrochloride. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenicity** of C.I. Basic Red 9 monohydrochloride for male and female F344/N rats and for male and female B6C3F$_1$ mice. In male rats, C.I. Basic Red 9 monohydrochloride caused squamous cell carcinomas, trichoepitheliomas and sebaceous adenomas of the skin, subcutaneous fibromas, thyroid gland follicular cell adenomas and follicular cell carcinomas, Zymbal gland carcinomas, and hepatocellular carcinomas. In female rats, C.I. Basic Red 9 monohydrochloride caused subcutaneous fibromas, thyroid gland follicular cell adenomas or carcinomas (combined), and Zymbal gland carcinomas. In male mice, C.I. Basic Red 9 monohydrochloride caused hepatocellular carcinomas. In female mice, C.I. Basic Red 9 monohydrochloride caused hepatocellular carcinomas and adrenal gland pheochromocytomas or malignant pheochromocytomas (combined). Exposure to C.I. Basic Red 9 monohydrochloride also may have been related to increased incidences of mammary gland tumors in female rats and hematopoietic system tumors in female mice.

*Categories of evidence of carcinogenicity are defined in the Note to the Reader on page 2.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of C.I. Basic Red 9 Monohydrochloride is based on the 13-week studies that began in May 1978 and ended in August 1978, on the 1-year studies that began in June 1979 and ended in June 1980, and on the 2-year studies that began in June 1979 and ended in June 1981.

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The members of the Peer Review Panel who evaluated the draft Technical Report on C.I. Basic Red 9 monohydrochloride on March 29, 1985, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF C. I. BASIC RED 9 MONOHYDROCHLORIDE

On March 29, 1985, the draft Technical Report on the toxicology and carcinogenesis studies of C.I. Basic Red 9 monohydrochloride received 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 9:00 a.m. in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. Harper, a principal reviewer, agreed with the conclusions . He commented on the poor survival but said that, based on the 13-week studies, the doses selected seemed justified. Dr. Harper said that more flexibility in the design protocol would allow discontinuing or adjusting dosing when there is an obvious trend of toxicity or decreased survival.

As a second principal reviewer, Dr. Kociba agreed in principle with the conclusions, although he questioned the association of increased incidences of bile duct tumors in male rats and of mammary gland tumors in female rats with chemical exposure. With regard to the bile duct tumors, Dr. W. Eastin, NTP, said one was diagnosed as a carcinoma and the other two were difficult to diagnose and mention of bile duct tumors would be deleted. Concerning mammary gland tumors, Dr. Kociba noted that combining fibroadenomas with adenomas and adenocarcinomas was a departure from NTP guidelines. Dr. E. McConnell, NTP, said this was a departure reflecting more recent NTP experiences that indicate occasional occurrence of fibroadenomas and malignant tumors within the same tissue and some evidence that malignant tumors can arise from fibroadenomas. Dr. Kociba stated that thyroid gland function measurements in the short-term studies may have helped select doses for the 2-year studies.

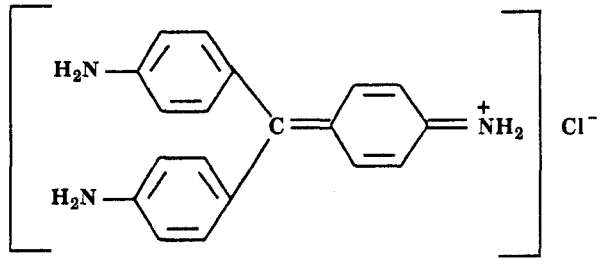
As a third principal reviewer, Dr. Purchase agreed with the conclusions in male and female rats but thought that the categorization of the findings in male and female mice should be some evidence of carcinogenicity. He said the reduction in body weight gain, the compound-induced mortality, and possibly compromised health of surviving mice made these conclusions suspect. Dr. Swenberg supported these comments for male mice in view of a high historical rate and variability for liver tumors but agreed with clear evidence of carcinogenicity in female mice. Dr. Harper noted that the liver carcinoma rates in males at both the low dose (40%) and the high dose (54%) were above the highest historical rate of 36%. Dr. J. Huff, NTP, agreed and added that the findings in both male and female mice were supportive.

Dr. Purchase questioned the positive findings reported in the mutagenicity studies, noting the marginally positive increase for mutations in *Salmonella typhimurium* and for sister-chromatid exchanges (SCE's) in Chinese hamster ovary cells along with an incomplete experimental design and/or reporting in the latter system. He said the guidelines of the United Kingdom Environmental Mutagen Society recommended a doubling of the sister-chromatid exchange incidence as being necessary for a positive effect. Dr. E. Zeigler, NIEHS, replied that regardless of the statistics used there was a strong positive response in *S. typhimurium* whereas in Chinese hamster ovary cells, the chemical was studied up to a concentration showing toxicity and there were dose-related increases in SCE's that were 30% above background for two of the three doses. Dr. Tannenbaum said the differences measured were statistically significant. Dr. Hook suggested that the discussion on the interpretation of the mutagenicity data be expanded. [See p. 19.]

Dr. Harper moved that the conclusion of clear evidence of carcinogenicity for male and female rats and female mice be accepted as written. Dr. Perera seconded the motion, and it was approved unanimously. Dr. Harper then moved that the conclusion of clear evidence of carcinogenicity for male mice be accepted as written. Dr. Hooper seconded the motion, and it was approved with six affirmative votes; there were four negative votes (Dr. Kotelchuck, Dr. Purchase, Dr. Swenberg, and Dr. Tannenbaum).

I. INTRODUCTION

I. INTRODUCTION



C.I. BASIC RED 9 MONOHYDROCHLORIDE

$C_{19}H_{17}N_3 \cdot HCl$

Molecular Weight: 323.8 **CAS No. 569-61-9**

**(Pararosaniline;
Benzenamine 4-((4-aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl)- monohydrochloride;
Paramagenta)**

C.I. Basic Red 9 monohydrochloride belongs to the triphenylmethane class of triarylmethane dyes. These brilliant dyes may be applied to a wide range of substrates. (For a review of triphenylmethane dyes, see Bannister and Elliott, 1983.) In 1972, 3,800 metric tons (3.8×10^9 g) of triarylmethane dyes were manufactured, an amount thought to represent about 4% of the total dyestuff production in the United States (USITC, 1972). The 1977 production estimate for C.I. Basic Red 9 monohydrochloride alone was reported to be 1-10 million pounds (4.5×10^8 g to 4.5×10^9 g) per year in the United States (USEPA, 1980). C.I. Basic Red 9 monohydrochloride (as a component of magenta) is used to color textiles, leather, and paper and as a biologic stain (IARC, 1974).

The basic fuchsins (C.I. Basic Red 9 monohydrochloride and related dyes) have been described as among the most powerful nuclear dyes (Witterholt, 1969). Basic fuchsin is a mixture of three closely related 4,4',4''-triaminotriarylmethane dyes (C.I. Basic Red 9 monohydrochloride, rosaniline, and Magenta II) in the form of their monohydrochloride salts (IARC, 1974; Lillie, 1977). The relative amounts of each depend on the desired color. For example, magenta as a commercial product is a mixture containing mostly rosaniline, some C.I. Basic Red 9 monohydrochloride, and a small amount of Magenta II (Witterholt, 1969).

The only evidence for the carcinogenicity of C.I. Basic Red 9 monohydrochloride found in the literature is the induction of sarcomas at the injection site following subcutaneous administration in rats (Druckey et al., 1956). Intragastric administration of 600 mg/kg C.I. Basic Red 9 monohydrochloride in 0.9% sodium chloride to 40 male and 40 female Sprague-Dawley rats two times per week for life beginning at 12 weeks of age resulted in rapid weight loss, diarrhea, and short average survival, showing that C.I. Basic Red 9 monohydrochloride was toxic and not well tolerated (Ketkar and Mohr, 1982). Dosing was discontinued for 1 week after week 2 and week 12 of exposure, and at week 18 the dose was halved. Average survival time for dosed males was 70 ± 27 days; for dosed females, 69 ± 29 days; and for controls, 104 ± 11 days for males and 92 ± 22 days for females. Thus, the study could not be considered an adequate test for carcinogenic potential. One adenoma and one carcinoma of the thyroid gland were observed in dosed male rats; none was observed in the controls.

In mice, 0.2 ml of magenta (methylated C.I. Basic Red 9) as a 3% suspension in arachis oil was given by stomach tube (12 mg/kg) to 30 males and 30 females for 52 weeks (Bonser et al., 1956). After 52 weeks, dosing was stopped and the mice were allowed to live as long as possible. Seven males and 13 females died before 90 weeks on study. The authors reported one hepatoma at week 101 in one female and concluded that magenta was without carcinogenic activity in this experiment. No differences (compared with controls) were observed in dosed mice in lifetime exposure studies by feeding, gavage, subcutaneous injection, or cutaneous

applications when leucoparafuchsin (the reduced form of C.I. Basic Red 9) was used (Malyugina and Prokof'yeva, 1957; Prokof'yeva, 1973; Prokof'yeva and Zabezhinskiy, 1976).

When 0, 300, or 600 mg/kg C.I. Basic Red 9 monohydrochloride in 0.9% sodium chloride was intragastrically administered two times per week for life to groups of 40 male and 40 female 12-week-old Syrian golden hamsters, the majority of the high dose animals (600 mg/kg) died within the first 10 weeks (Green et al., 1979). These authors concluded that the 300 mg/kg dose had no effect on body weight or survival. Fifty percent of the males in both the dosed (300 mg/kg) and control groups survived to 56 weeks; dosed (300 mg/kg) females survived to week 32 versus week 40 for controls.

The evaluation of the carcinogenic risk to humans of magenta (including C.I. Basic Red 9 monohydrochloride) was the subject of an IARC monograph (IARC, 1974). The IARC conclusions cited the Druckey et al. (1956) study in rats (already discussed) as providing the only evidence of carcinogenicity in animals and one epidemiologic study indicating a carcinogenic risk to workers involved in the manufacture of magenta (of which C.I. Basic Red 9 monohydrochloride is a component) (Case and Pearson, 1954).

C.I. Basic Red 9 monohydrochloride was not mutagenic in a variety of strains of *Salmonella typhimurium* in the presence or absence of mouse or rat liver S9 when tested according to the standard plate-incorporation protocol (McCann et al., 1975; Dunkel, 1979; Rosenkranz and Poirier, 1979; Simmon, 1979a; DeFlora, 1981) or by host-mediated assay in mice (Simmon et al., 1979). However, C.I. Basic Red 9 monohydrochloride was mutagenic in the plate-incorporation assay in the presence of Aroclor 1254-induced hamster liver S9 (Dunkel, 1979). Using the preincubation protocol, the NTP found that C.I. Basic Red 9 monohydrochloride was mutagenic in strains TA100 and TA98 of *S. typhimurium* in the presence of Aroclor 1254-induced male rat or male hamster liver S9 (Appendix G, Table G1). The urine of dosed B6C3F$_1$ mice in the present 2-year studies was found to be mutagenic (Haworth et al., 1981). The urine from male mice was mutagenic in strains TA98 and TA100, and the urine from female mice was mutagenic in strain TA98. The addition of rat or hamster liver S9 increased the mutagenicity of the urine.

C.I. Basic Red 9 monohydrochloride also induced DNA damage in *Escherichia coli* (Rosenkranz and Poirier, 1979), but it did not induce mitotic recombination in yeast (Simmon, 1979b). The NTP found that C.I. Basic Red 9 monohydrochloride was mutagenic in the L5178Y/TK$^{+/-}$ mouse lymphoma assay in the presence or absence of Aroclor 1254-induced male rat liver S9 (Appendix G, Tables G2 and G3). Although C.I. Basic Red 9 monohydrochloride did not induce chromosomal aberrations in Chinese hamster ovary cells, it did induce sister-chromatid exchanges in the presence of Aroclor 1254-induced male rat liver S9 (Tables G4 and G5). The level of induction (20%-30% over background) is highly significant in the system being used (Galloway et al., 1985). The difference in doses used with the activation versus nonactivation systems is due to treatment procedure; i.e., in the former, cells are treated for only 2 hours whereas in the latter they are exposed for 26 hours. C.I. Basic Red 9 monohydrochloride was positive for DNA damage in the in vitro alkaline elution assay for single-stranded DNA with V79 cells (Swenberg et al., 1976). In addition, the NTP found that C.I. Basic Red 9 monohydrochloride induced unscheduled DNA synthesis in rat hepatocytes in vitro (Table G6). In summary, C.I. Basic Red 9 monohydrochloride is mutagenic and causes DNA damage in bacteria and mammalian cells; it induces sister-chromatid exchanges; and it produces mutagenic urine in mice that have been exposed to C.I. Basic Red 9 monohydrochloride in feed.

As an aromatic amine, C.I. Basic Red 9 monohydrochloride may form potentially carcinogenic metabolites by N-hydroxylation. It is structurally related to the known carcinogens 4,4'-methylenebis(2-methylaniline) and 4,4'-methylenebis(2-chloroaniline) (Stula et al., 1975, 1977, 1978). The compound was selected for study because it is produced in large volume with considerable potential for human exposure and because it is structurally related to known carcinogens and is a component of a mixture known to be carcinogenic.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

THIRTEEN-WEEK STUDIES

FIFTY-TWO-WEEK STUDIES

TWO-YEAR STUDIES

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

PROCUREMENT AND CHARACTERIZATION OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

C.I. Basic Red 9 monohydrochloride was obtained in two batches (Table 1). Purity and identity analyses were conducted at Midwest Research Institute (Kansas City, Missouri) (Appendix H).

Both lots were identified as C.I. Basic Red 9 monohydrochloride by spectroscopy. Infrared and nuclear magnetic resonance spectra were consistent with those expected for the structure of C.I. Basic Red 9 monohydrochloride and with literature data; ultraviolet/visible spectra were consistent with its structure.

For lot no. PO1340, cumulative data from elemental analysis, nonaqueous amine titration, Karl Fischer water analysis, and thin-layer and high-performance liquid chromatography indicated a purity of approximately 93% with the major impurity being identified as water (approximately 9%). Cumulative data for lot no. A7X from elemental analysis, nonaqueous amine titration, Karl Fischer water analysis, and thin-layer and high-performance liquid chromatography indicated a purity of approximately 99%, with water again being identified as the major impurity (approximately 0.5%). A sample of this lot was sent to Thermo Electron Corporation for analysis of possible nitrosamine impurities. High-performance liquid chromatography/thermal energy analyzer analysis of the test sample indicated a single, nonpolar nitrosamine at a concentration of 0.5 ppm; the nitrosamine was not identified.

C.I. Basic Red 9 monohydrochloride was stored in the dark at 4° C. Results of periodic bulk reanalyses of lot no. A7X at EG&G Mason Research Institute by thin-layer chromatography and infrared spectroscopy indicated that no degradation of the bulk chemical occurred during the course of the 2-year studies.

TABLE 1. IDENTITY AND SOURCE OF LOTS USED IN THE FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Thirteen-Week Studies	Fifty-Two-Week Studies	Two-Year Studies
Lot Numbers	PO1340	PO1340 until 9/23/79 (first 16 weeks of study); A7X from 9/23/79 until the end of the study	Same as 52-wk studies
Supplier	Pfaltz and Bauer (Stamford, CT)	PO1340--Pfaltz and Bauer (Stamford, CT); A7X--Fisher Scientific (St. Louis, MO)	Same as 52-wk studies

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

Formulated diets were prepared as described in Table 2 and Appendix H. C.I. Basic Red 9 monohydrochloride was found to be stable in feed for 2 weeks when stored at 5° C or below (Appendix I). Test diets were stored at 4° C for no longer than 14 days.

Formulations of C.I. Basic Red 9 monohydrochloride in feed were analyzed periodically by the testing and referee laboratories to confirm test chemical content. The analytical method included a methanolic extraction as a purification step and either high-performance liquid chromatographic assay (testing laboratory) or ultraviolet/visible spectroscopy (referee laboratory) as the quantitation step (Appendix J). All 35 mixes analyzed for the 2-year studies were formulated within ± 10% of the target concentration (Table 3; Appendix K).

TABLE 2. PREPARATION AND STORAGE OF FORMULATED DIETS IN THE FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Thirteen-Week Studies	Fifty-Two-Week Studies	Two-Year Studies
Preparation	C. I. Basic Red 9 monohydrochloride was weighed into a plastic bag and an aliquot of meal added; the bag was shaken until a homogenous mixture was obtained; the premix was sandwiched between the remaining meal in a Patterson-Kelly® twin-shell V-blender and mixed for 20 min	A premix of C. I. Basic Red 9 monohydrochloride was homogenized in a mortar with a pestle, layered between appropriate amounts of feed, and mixed for 20 min in an 8-qt Patterson-Kelly® V-blender with no intensifier bar	Same as 52-wk studies
Maximum Storage Time	1 wk	14 d	Same as 52-wk studies
Storage Conditions	Sealed in double plastic bags, stored in the dark	In double plastic bags within plastic buckets at 4° C	In double plastic bags within plastic buckets at 4° C

TABLE 3. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Determined Concentration for Target Concentration of		
	500 ppm	1,000 ppm	2,000 ppm
Mean (ppm)	513	1,048	2,062
Standard deviation	25.1	51.9	93.8
Coefficent of variation (percent)	4.9	5.0	4.5
Range (ppm)	460-540	950-1,100	1,900-2,200
Number of samples	12	12	11

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxicity of C.I. Basic Red 9 monohydrochloride and to determine the concentrations to be used in the 2-year studies. Four- to five-week-old male and F344/N female rats and 5- to 6-week-old B6C3F$_1$ mice were obtained from Harlan Industries. Rats were quarantined for 3 weeks and mice for 2 weeks. Animals were then randomized by weight and assigned to test groups so that average cage weights were approximately equal for all animals of the same sex and species. Diets containing 0, 250, 500, 1,000, 2,000, or 4,000 ppm C.I. Basic Red 9 monohydrochloride were fed for 13 weeks to groups of 10 rats and mice of each sex.

Rats and mice were housed five per cage in polycarbonate cages. Test diets, control diets, and water were available ad libitum. Further experimental details are summarized in Table 4. Animals were checked twice daily, and moribund animals were killed. Feed consumption was measured weekly by cage. Animal weights were recorded weekly. At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 4.

FIFTY-TWO-WEEK STUDIES

Fifty-two-week studies were conducted to observe the effects of C.I. Basic Red 9 monohydrochloride on the thyroid gland of rats. Four- to five-week-old male and female F344/N rats were obtained from Charles River Breeding Laboratories, observed for 2 weeks, and then randomized by weight and assigned to test groups so that the average cage weights were approximately equal for all animals of the same sex. Diets containing 0 or 1,000 ppm C.I. Basic Red 9 monohydrochloride were fed for 52 weeks to groups of 10 female rats. Groups of 10 male rats received 0 or 2,000 ppm.

Rats were housed five per cage in polycarbonate cages. Test diets, control diets, and water were available ad libitum. Further experimental details are summarized in Table 4. Animals were checked twice daily. Animal weights were recorded once per week for the first 13 weeks and once every 4 weeks thereafter. The thyroid glands of all animals were palpated once per month. Blood was collected from the orbital sinus during the 1st and 2nd weeks of quarantine and at weeks 13, 26, 39, and 52. Serum thyroxin levels were determined by radioimmunoassay with a SPAC® T4 RIA kit from Malinckrodt (St. Louis, Missouri) (Appendix N).

TWO-YEAR STUTIDES

Study Design

Diets containing 0, 1,000, or 2,000 ppm C.I. Basic Red 9 monohydrochloride were fed to groups of 50 male rats for 103 weeks. Groups of 50 female rats and groups of 50 mice of each sex were fed diets containing 0, 500, or 1,000 ppm for 103 weeks.

Source and Specifications of Animals

The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female, × C3H/HeN MTV$^-$, male) mice used in this study were produced under strict barrier conditions at Charles River Breeding Laboratories under a contract to the Carcinogenesis Program. Breeding starts for the foundation colony at the production facility originated at the National Institutes of Health Repository. Animals shipped for testing were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Male and female F344/N rats and female B6C3F$_1$ mice were shipped to the testing laboratory at 4 weeks of age. The animals were quarantined at the testing facility for 2 weeks. Male B6C3F$_1$ mice were shipped to the testing laboratory at 5-6 weeks of age and quarantined at the testing facility for 26 days. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. The rats and female mice were placed on study at 6 weeks of age and male mice, at 9-10 weeks of age. The health of the animals was monitored during the course of the study according to the protocols of the NTP Sentinel Animal Program (Appendix L).

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to

TABLE 4. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Thirteen-Week Studies	Fifty-Two-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN			
Size of Test Groups	10 males and10 females of each species	10 male and 10 female rats	50 males and 50 females of each species
Doses	0, 250, 500, 1,000, 2,000, or 4,000 ppm C.I. Basic Red 9 monohydrochloride in feed	0, 1,000 (females only), or 2,000 (males only) ppm C.I. Basic Red 9 monohydrochloride in feed	Male rats--0, 1,000, or 2,000 ppm; female rats and male and female mice--0, 500, or 1,000 ppm C. I. Basic Red 9 monohydrochloride in feed
Date of First Dose	5/17/78	6/4/79	Rats--6/4/79; mice--6/21/79 (female), 4/21/80 (restarted male)
Date of Last Dose	8/16/78	6/10/80	Rats--5/27/81; female mice--6/14/81; male mice--4/12/82
Duration of Dosing	13 wk	52 wk	103 wk
Type and Frequency of Observation	Observed 2 × d; weight and feed consumption measured 1 × wk	Observed 2 × d; weighed 1 × wk for 13 wk, 1 × mo thereafter; thyroid glands palpated 1 × mo	Observed 2 × d; weighed 1 × wk for 13 wk, 1 × 4 wk until 3/25/81, 2 × mo thereafter
Necropsy and Histologic Examination	Necropsy performed on all animals; histologic exam performed on control and 4,000-ppm groups; the following tissues were examined: gross lesions and tissue masses, skin, mandibular and mesenteric lymph nodes, mammary gland, salivary gland, thigh muscle, lungs and mainstem bronchi, heart, thyroid gland, parathyroids, esophagus, stomach, duodenum, colon, jejunum, ileum, cecum, rectum, gallbladder (mice), liver, sciatic nerve, sternebrae, costochondral junction, thymus, larynx, trachea, pancreas, spleen, kidneys, adrenal glands, urinary bladder, nasal cavity, brain, pituitary gland, spinal cord, eyes, seminal vesicles/prostate/testes or ovaries/uterus; thyroid gland and pituitary gland of 2,000-ppm rats and 1,000-ppm female rats were also examined histologically	Blood for thyroxin determinations was taken at 0, 13, 26, 39, and 52 wk; necropsy performed on all animals; tissues examined same as in 13-wk studies; thyroid gland and pituitary gland were examined histologically	Necropsy performed on all animals; histologic exam performed on all animals; tissues examined include: gross lesions and tissue masses, skin, mandibular and mesenteric lymph node, mammary gland, salivary gland, thigh muscle, lungs and mainstem bronchi, heart, thyroid gland, parathyroids, esophagus, stomach, small intestine, colon, gallbladder (mice), liver, sternebrae (including marrow), costochondral junction, thymus, larynx, trachea, pancreas, spleen, kidneys, adrenal glands, urinary bladder, brain, pituitary gland, spinal cord (if neurologic signs present), eyes (if grossly abnormal), seminal vesicles/prostate/testes or ovaries/uterus
ANIMALS AND ANIMAL MAINTENANCE			
Strain and Species	F344/N rats; B6C3F$_1$ mice	F344/N rats	F/344/N rats; B6C3F$_1$ mice
Animal Source	Harlan Industries (Indianapolis, IN)	Charles River Breeding Laboratories (Portage, MI)	Rats and female mice--Charles River Breeding Laboratories (Portage, MI); male mice--Charles River Breeding Laboratories (Kingston, NY)

TABLE 4. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Thirteen-Week Studies	Fifty-Two-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)			
Testing Laboratory	EG&G Mason Research Institute	Same as 13-wk studies	Same as 13-wk studies
Time Held Before Test	Rats--21 d; mice--14 d	14 d	Rats--14 d; female mice--16 d male mice--26 d
Age When Placed on Study	7-9 wk	6-7 wk	Rats and female mice--6-7 wk; male mice--9-10 wk
Age When Killed	Rats--21-22 wk; mice--20-21 wk	59-60 wk	Rats, female mice--110-113 wk; male mice--113-115 wk
Necropsy Dates	Rats--8/24/78-8/28/78; mice--8/17/78-8/23/78	6/11/80-6/12/80	Rats--6/4/81-6/17/81; female mice--6/22/81-6/23/81; male mice--4/20/82-4/29/82
Method of Animal Distribution	Randomized by weight so that average body weights for each group were approximately equal	Same as 13-wk studies	Assigned to cages, according to a table of random numbers; cages then assigned to groups according to another table of random numbers
Method of Animal Identification	Ear punch	Ear punch	Ear punch
Feed	Ground Wayne Lab Blox® (Allied Mills, Chicago, IL); freely available	Same as 13-wk studies	Same as 13-wk studies
Bedding	Aspen® bed (American Excelsior, Baltimore, MD)	Same as 13-wk studies; and Betta Chips®; Agway Corp. (Syracuse, NY)	Same as 13-wk studies
Water	Automatic watering system (Edstrom Industries, Waterford, WI); freely available	Same as 13-wk studies	Same as 13-wk studies
Cages	Polycarbonate Lab Products (Maywood, NJ)	Same as 13-wk studies	Same as 13-wk studies
Cage Filters	Rats--Webrex® nonwoven (Negus Container Co., Madison, WI); mice--spun-bonded bonnets (Lab Products, Rochelle Park, NJ)	Nonwoven (Lab Products, Rochelle Park, NJ, or Snow Filtration Co., Cincinnati, OH)	Same as 52-wk studies
Animals per Cage	5	5	Rats and female mice--5; male mice--1
Other Chemicals on Test in Same Room	None	None	None
Animal Room Environment	Temp--20°-30°C; humidity--34%-81%; fluorescent light 12 h/d; 10 room air changes/h	Temp--19°-30° C; humidity--20%-95%; fluorescent light 12 h/d; 10-15 room air changes/h	Temp--18°-27° C; humidity--20%-95%; fluorescent light 12 h/d; 12 room air changes/h

produce the hybrid B6C3F$_1$ test animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoretograms that demonstrate phenotype expressions of known genetic loci.

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

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

Animal Maintenance

Rats and female mice were housed five per cage. Male mice were initially housed five per cage, but fighting among cagemates caused a significant number of deaths in all test groups, including controls. The study in male mice was restarted, and the male mice were housed individually. Feed and water were available ad libitum. Details of animal maintenance are summarized in Table 4.

Clinical Examinations and Pathology

All animals were observed twice daily, and clinical signs were recorded once per week. Body weights by cage were recorded once per week for the first 12 weeks of the study and once per month thereafter. Mean body weights were calculated for each group. Feed consumption was recorded every 4 weeks. Moribund animals were killed, as were animals that survived to the end of the study. A necropsy was performed on all animals, including those found dead unless they were excessively autolyzed or cannibalized. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study in each group.

Examinations for grossly visible lesions were performed on major tissues or organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues examined microscopically are listed in Table 4.

When the pathology examination was completed, the slides, individual animal data records, and summary tables were sent to an independent quality assurance laboratory. Individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assurance pathologist. Slides of all target tissues and those about which the original and quality assurance pathologists disagreed were submitted to the Chairperson of the Pathology Working Group (PWG) for evaluation. Representative coded slides selected by the Chairperson were reviewed by PWG pathologists, who reached a consensus and compared their findings with the original and quality assurance diagnoses. When diagnostic differences were found, the PWG sent the appropriate slides and comments to the original pathologist for review. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent evaluations, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Nonneoplastic lesions are not examined routinely by the quality assurance pathologist or PWG. Certain nonneoplastic findings are reviewed by the quality assurance pathologist and PWG if they are considered part of the toxic response to a chemical or if they are deemed of special interest.

Statistical Methods

Data Recording: 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, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. All reported P values for the survival analysis are two-sided.

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

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data. The two that adjust for intercurrent mortality employ the classical method for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high dose and low dose groups with controls and tests for overall dose-response trends.

For studies in which compound administration has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data depends on the extent to which the tumor under consideration is regarded as being the cause of death. All reported P values for tumor analyses are one-sided.

Life Table Analyses--The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analyses--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals on which a necropsy was actually performed during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.)

Unadjusted Analyses--Primarily, survival-adjusted methods are used to evaluate tumor incidence. In addition, the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) were carried out. These two tests are

based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

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

III. RESULTS

RATS

THIRTEEN-WEEK STUDIES

FIFTY-TWO-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

MICE

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

THIRTEEN-WEEK STUDIES

One male and two female rats that received 4,000 ppm C.I. Basic Red 9 monohydrochloride died before the end of the studies (Table 5). Final mean body weights relative to those of the controls were 14% and 37% lower for females that received 2,000 ppm or 4,000 ppm and 40% lower for males that received 4,000 ppm.

At necropsy, enlarged thyroid glands were found in 8/10 male and 8/10 female rats that received 4,000 ppm and in 3/10 female rats that received 2,000 ppm. Adenomatous goiter was seen in 9/10 males and 9/9 females that received 4,000 ppm. Diffuse hyperplasia of the thyroid gland occurred in 1/10 male rats that received 4,000 ppm and in 7/10 female rats that received 2,000 ppm, as compared with 0/10 male controls and 0/9 female controls. Adenomatous goiter was characterized by overdistended cells with an excess of colloid, papillary infolding of follicular epithelium, retrogressive changes such as intrafollicular hemorrhage, and capsular and interstitial fibrosis. In the high dose male rat with diffuse follicular hyperplasia, the follicular cells were columnar with focal piling up of cells. Colloid was not found in follicular lumens.

Pituitary basophil hypertrophy was seen with H&E and Aldehyde Thionine PAS staining. The hypertrophied cells were identified as basophils by the presence of dark blue cytoplasmic granules after staining. Basophil hypertrophy was found in 5/7 male and 8/9 female rats that received 4,000 ppm and in 1/9 male and 1/10 female rats that received 2,000 ppm, as compared with none in the 9 male and 8 female controls examined.

A fatty change in the liver was found in 1/10 male and 4/10 female rats that received 4,000 ppm but not in rats that received lower concentrations.

Dose Selection Rationale: Because lower mean body weights were observed at higher concentrations, doses selected for rats for the 2-year studies were 1,000 or 2,000 ppm C.I. Basic Red 9 monohydrochloride for males and 500 or 1,000 ppm for females.

TABLE 5. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Concentration (ppm)	Survival (a)	Mean Body Weight (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 4	Week 12
MALE							
0	10/10	136 ± 3	281 ± 6	+145 ± 5	--	112	85
250	10/10	135 ± 2	265 ± 7	+130 ± 6	94.3	111	87
500	10/10	135 ± 3	290 ± 8	+155 ± 7	103.2	102	72
1,000	10/10	136 ± 3	286 ± 8	+150 ± 6	101.8	95	72
2,000	10/10	136 ± 3	274 ± 7	+138 ± 6	97.5	96	74
4,000	(e) 9/10	137 ± 3	169 ± 6	+ 32 ± 5	60.1	84	74
FEMALE							
0	10/10	106 ± 3	178 ± 4	+ 72 ± 2	--	98	90
250	10/10	106 ± 3	170 ± 7	+ 64 ± 5	95.5	96	71
500	10/10	107 ± 3	170 ± 5	+ 63 ± 3	95.5	95	74
1,000	10/10	107 ± 3	170 ± 4	+ 63 ± 2	95.5	93	70
2,000	10/10	106 ± 2	153 ± 3	+ 47 ± 2	86.0	147	72
4,000	(f) 8/10	107 ± 4	112 ± 4	+ 6 ± 4	63.0	86	82

(a) Number surviving/number initially in the group
(b) Initial mean body weight ± standard error of the mean of all animals in the group. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors of the group ± standard error of the mean
(d) Grams of feed consumed per kilogram of body weight per day
(e) Week of death: 3
(f) Week of death: 7, 9

FIFTY-TWO-WEEK STUDIES

One control male and one male that received 2,000 ppm died (Table 6). Final mean body weights were 13% lower than that of controls for dosed male rats and 9% lower for dosed female rats. Of male rats exposed at 2,000 ppm, 1/10 had hyperplasia, 1/10 had an adenoma, and 1/10 had a carcinoma of the follicular epithelium of the thyroid gland at the end of 1 year. One of 10 males exposed at 2,000 ppm had thyroid gland follicular cysts; this lesion also occurred in 2/10 females exposed at 1,000 ppm.

The mean absolute thyroid gland weight of dosed males was 1.40 times that of the controls, and the mean relative thyroid gland weight of the dosed males was 1.69 times that of the controls (Table 7). In contrast, the relative thyroid gland weight of the female rats was only 1.13 times that of the controls.

The concentration of thyroxin in dosed rats was significantly lower than that of the controls by week 13 in males and by week 52 in females (Table 8).

Four of 10 males had fatty metamorphosis of the liver; 2 of the males with fatty metamorphosis had focal necrosis of the liver, and 1 of these had a neoplastic nodule.

TABLE 6. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FIFTY-TWO-WEEK FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Concentration (ppm)	Survival	Mean Body Weight (grams)			Final Body Weight Relative to Controls (percent)
		Initial	Final (a)	Change	
MALE					
0	9/10	129	472	343	--
2,000	9/10	122	409	287	86.7
FEMALE					
0	10/10	96	264	168	--
1,000	10/10	95	240	145	90.9

(a) Weight at final monthly weighing

TABLE 7. RELATIVE THYROID GLAND WEIGHTS OF RATS IN THE FIFTY-TWO-WEEK FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Concentration (ppm)	Mean Body Weight ± SD (a) (grams)	No. of Animals	Mean Thyroid Gland Weight ± SD (grams × 10^{-3})	Ratio (Thyroid Gland Wt:Body Wt) (× 10^{-5})
MALE				
0	457 ± 22	9	28.3 ± 4.6	6.18 ±0.90
2,000	(b) 389 ± 56	(c) 7	(b) 39.6 ± 1.3	(b) 10.42 ± 2.01
Ratio (dosed:control)	0.82		1.40	1.69
FEMALE				
0	254 ± 20	10	23.3 ± 4.1	9.23 ± 1.84
1,000	(b) 231 ± 18	10	24.3 ± 3.5	10.56 ± 1.67
Ratio (dosed:control)	0.91		1.04	1.13

(a) Mean body weight at necropsy ± standard deviation
(b) $P<0.05$ by two-sided *t*-test comparison with the controls
(c) Two animals with thyroid gland tumors were deleted from this group as outliers; thyroid gland weights: 98.8 mg and 61.6 mg.

TABLE 8. CONCENTRATIONS OF THYROXIN IN THE SERUM OF RATS IN THE FIFTY-TWO-WEEK FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Week	No. of Animals (a) (controls)	Thyroxin Level of Controls (b)	No. of Animals (dosed)	Thyroxin Level of Dosed Groups (b)	Ratio (c)	P Value (d)
MALE						
−1	10	5.34 ± 0.58	10	4.98 ± 0.49	0.93	0.13
0	10	3.74 ± 0.74	10	3.66 ± 0.72	0.98	0.38
13	9	4.19 ± 0.42	10	3.25 ± 0.64	0.78	0.002
26	8	4.28 ± 0.34	10	3.23 ± 0.68	0.75	<0.001
39	8	3.08 ± 0.33	10	1.99 ± 0.40	0.65	<0.001
52	8	3.30 ± 0.46	9	1.72 ± 0.59	0.52	<0.001
FEMALE						
−1	10	3.72 ± 0.49	10	4.09 ± 0.53	1.10	0.11
0	10	3.73 ± 0.34	10	3.48 ± 0.54	0.93	0.18
13	10	3.17 ± 0.60	10	2.90 ± 0.45	0.91	0.20
26	9	2.76 ± 0.52	10	2.20 ± 0.39	0.80	0.019
39	9	2.51 ± 0.53	10	2.27 ± 0.55	0.90	0.24
52	9	2.60 ± 0.56	10	1.66 ± 0.41	0.64	<0.001

(a) No serum sample was obtainable from one male and one female control after 13 weeks on study.
(b) Mean: micrograms/100 ml ± standard deviation; dietary concentration of C.I. Basic Red 9 monohydrochloride for males is 2,000 ppm and for females, 1,000 ppm.
(c) Ratio of thyroxin level in dosed groups to that in controls
(d) Two-tailed *t*-test comparison of the control and dosed groups

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of high dose rats of each sex were marginally lower than those of the controls (Table 9 and Figure 1). This effect appears to have been progressive. The average daily feed consumption per rat by low dose and high dose rats was 99% that of the controls for males and 96% and 97% for females (Appendix L, Tables L1 and L2). The average daily doses of C.I. Basic Red 9 monohydrochloride were approximately 49 and 103 mg/kg body weight for low dose and high dose male rats and 28 and 59 mg/kg body weight for low dose and high dose female rats.

TABLE 9. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Weeks on Study	Control		Low Dose			High Dose		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE			1,000 ppm			2,000 ppm		
0	111	50	108	97	50	110	99	50
1	152	50	152	100	50	146	96	50
2	187	50	187	100	50	178	95	50
3	212	50	209	99	50	200	94	50
4	229	50	229	100	50	219	96	50
5	247	50	246	100	50	237	96	50
6	256	50	255	100	50	248	97	50
7	266	50	263	99	50	258	97	50
8	277	50	267	96	50	261	94	50
9	288	50	279	97	50	273	95	50
10	300	50	292	97	50	285	95	50
11	307	50	299	97	50	293	95	50
12	300	50	287	96	50	282	94	50
16	341	50	335	98	50	322	94	49
20	358	50	347	97	50	342	96	47
24	377	50	367	97	50	359	95	47
28	394	50	380	96	50	373	95	47
32	396	50	390	98	50	385	97	47
36	415	50	401	97	50	392	94	47
40	426	50	412	97	50	400	94	47
44	434	50	422	97	50	407	94	46
48	441	50	430	98	50	414	94	46
52	446	50	434	97	50	416	93	46
56	447	50	438	98	49	418	94	46
60	442	50	433	98	49	411	93	44
64	448	48	439	98	48	415	93	41
68	450	48	435	97	48	408	91	38
72	450	48	435	97	48	398	88	35
76	454	47	437	96	48	409	90	28
80	461	47	448	97	48	421	91	24
84	460	45	447	97	47	413	90	20
88	462	45	449	97	44	403	87	14
92	467	43	449	96	40	415	89	11
94	--	--	--	--	--	408	--	9
96	460	41	447	97	34	379	82	7
98	--	--	--	--	--	393	--	4
100	453	39	439	97	34	396	87	2
102	--	--	--	--	--	388	--	2
104	435	37	430	99	31	--	--	--
FEMALE			500 ppm			1,000 ppm		
0	98	50	99	101	50	96	98	50
1	124	50	124	100	50	122	98	50
2	142	50	141	99	50	139	98	50
3	179	50	151	84	50	148	83	50
4	158	50	156	99	50	154	97	50
5	168	50	166	99	50	162	96	50
6	173	50	172	99	50	166	96	50
7	179	50	177	99	50	173	97	50
8	185	50	182	98	50	177	96	50
9	188	50	186	99	50	180	96	50
10	192	50	197	103	50	183	95	50
11	195	50	191	98	50	184	94	50
12	196	50	190	97	50	180	92	50
16	210	50	207	99	50	190	90	50
20	215	50	212	99	50	202	94	50
24	221	50	216	98	50	206	93	50
28	225	50	223	99	50	210	93	50
32	230	50	225	98	50	213	93	49
36	234	50	231	99	50	219	94	49
40	246	50	239	97	50	224	91	49
44	252	50	242	96	50	229	91	49
48	257	50	251	98	50	235	91	49
52	265	50	257	97	50	240	91	49
56	275	49	267	97	50	249	91	49
60	282	49	270	96	50	250	89	48
64	289	49	280	97	50	258	89	46
68	300	49	287	96	50	265	88	45
72	306	49	295	96	50	271	89	43
76	317	49	302	95	50	277	87	43
80	330	47	312	95	50	288	87	42
84	336	47	320	95	49	294	88	39
88	343	44	324	94	49	308	90	34
92	343	43	329	96	48	306	89	31
94	--	--	--	--	--	311	--	27
96	348	40	332	95	45	314	90	24
98	--	--	--	--	--	326	--	22
100	349	40	349	100	41	315	90	19
102	--	--	--	--	--	325	--	16
104	346	37	340	98	34	324	94	12

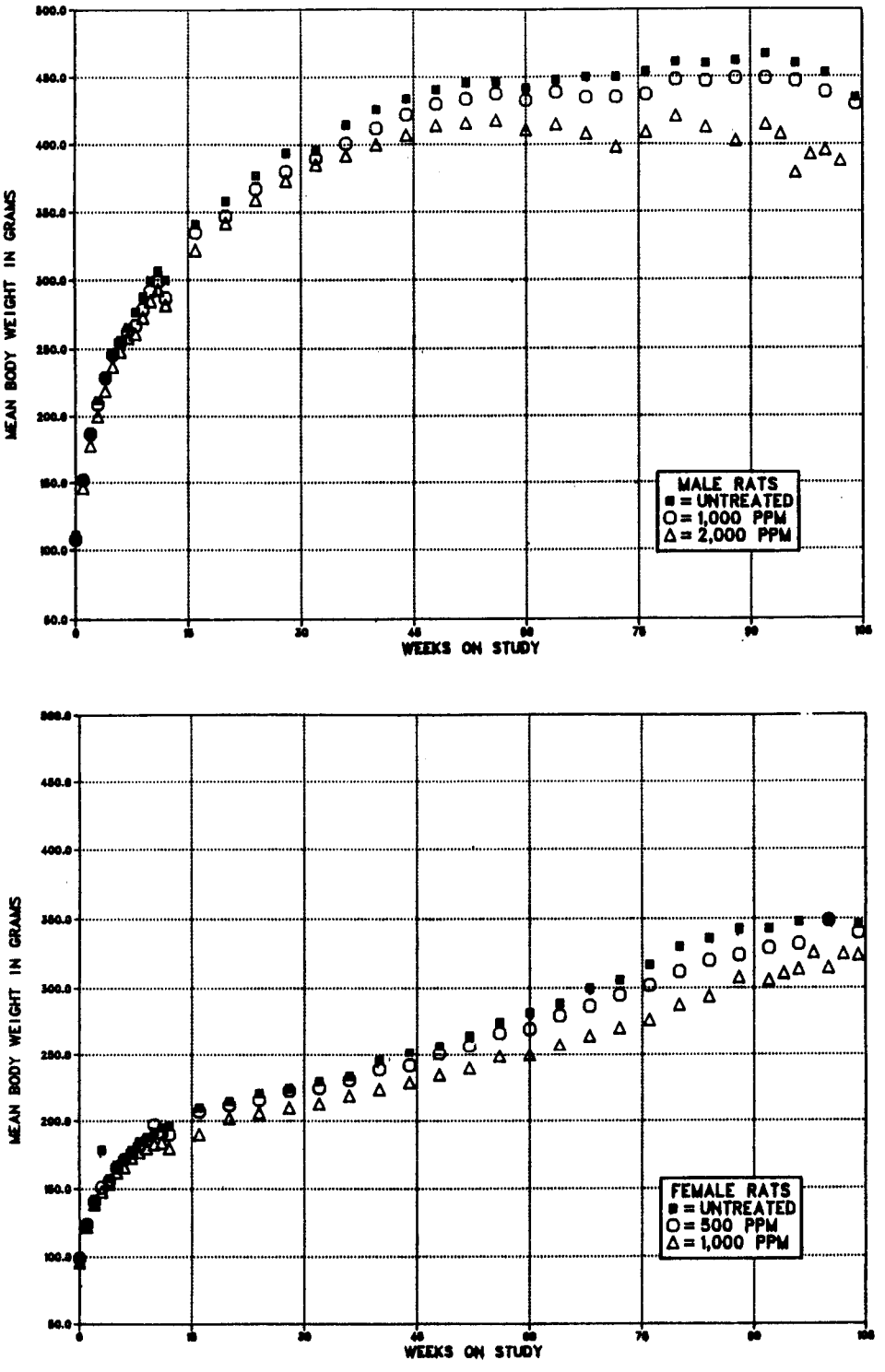


FIGURE 1. GROWTH CURVES FOR RATS FED DIETS CONTAINING C. I. BASIC RED 9 MONOHYDROCHLORIDE FOR TWO YEARS

Survival

Estimates of the probabilities of the survival of male and female rats fed diets containing C.I. Basic Red 9 monohydrochloride at the concentrations used in these studies and those of the controls are shown in the Kaplan and Meier curves in Figure 2. Survival of the high dose male and female rats was significantly lower than that of either the low dose or control groups ($P<0.001$) (Table 10). No high dose male rats survived to 104 weeks (see Table 9).

Pathology and Statistical Analyses of Results

This section describes significant or noteworthy changes in the incidence of rats with neoplastic or nonneoplastic lesions of the skin, subcutaneous tissue, mammary gland, thyroid gland, Zymbal gland, liver, urinary system, uterus, lung, and hematopoietic system. Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); Appendix A (Tables A3 and A4) also gives the survival and tumor status for individual male and female rats. Findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2). Appendix E (Tables E1 and E2) contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes). Historical incidences of tumors in control animals are listed in Appendix F.

The statistical analyses and interpretation of the tumor incidence data for high dose male and female rats were complicated by the marked reduction in survival in these groups when compared with that of the controls. In this situation, the incidental tumor test has relatively little sensitivity; hence, results of this test were not given major emphasis for rats, although, for completeness, they are included in Appendix E. Instead, in this section, the results of unadjusted analyses (Fisher exact test and Cochran-Armitage test) and life table analyses are presented.

A positive effect by both life table and unadjusted analyses was considered evidence that an increase in tumor incidence was related to chemical exposure, except when neoplasms were clearly recognized as the cause of death (life table analysis would be appropriate in this instance).

TABLE 10. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	Low Dose	High Dose
MALE (a)	**Control**	**1,000 ppm**	**2,000 ppm**
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	14	21	50
Killed at termination	33	28	0
Died during termination period	3	1	0
Survival P values (c)	<0.001	0.215	<0.001
FEMALE (a)	**Control**	**500 ppm**	**1,000 ppm**
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	13	15	36
Killed at termination	37	31	12
Died during termination period	0	4	2
Survival P values (c)	<0.001	0.967	<0.001

(a) Terminal kill period: male--weeks 105-106; female--weeks 104-106
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.

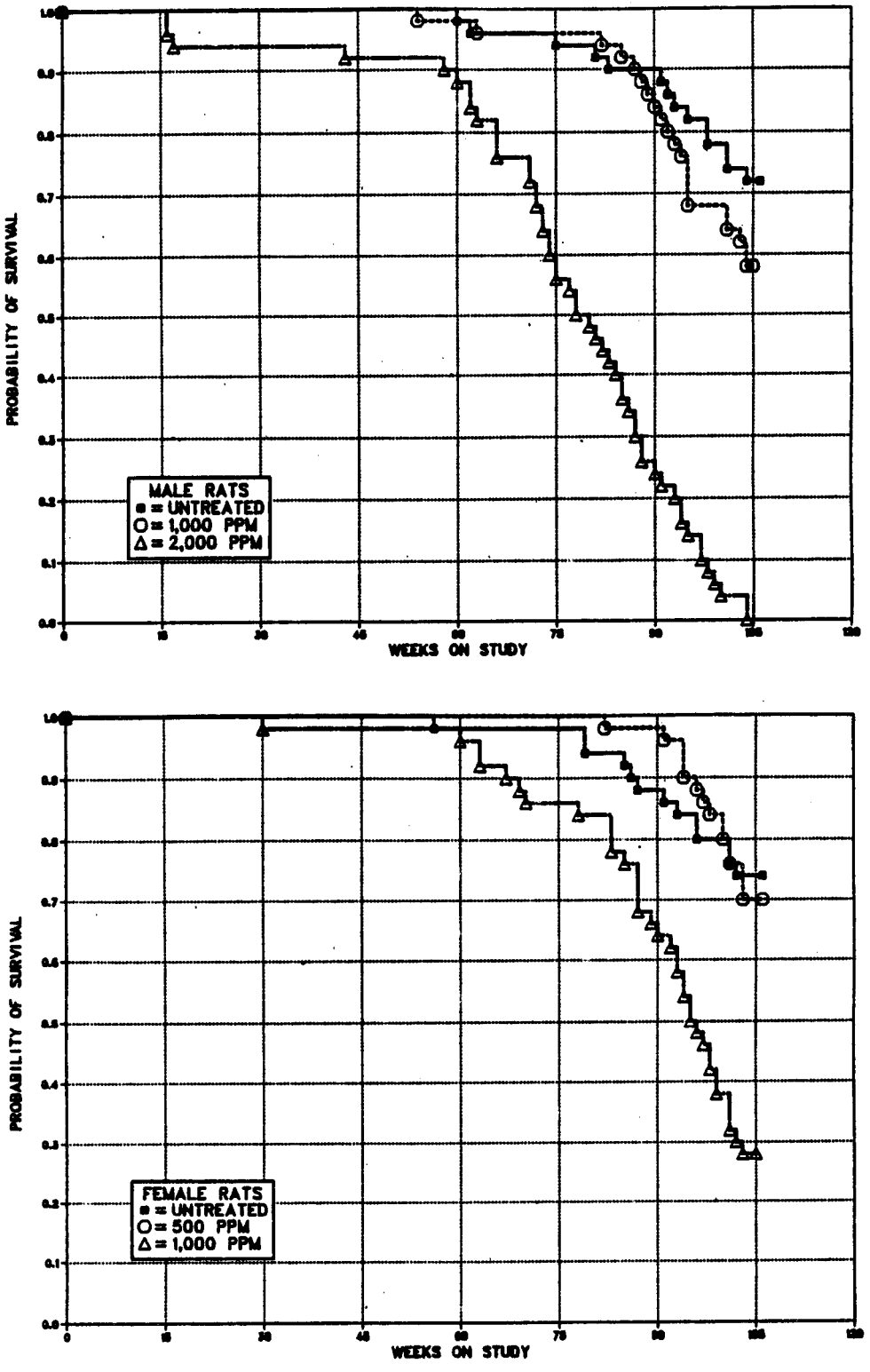


FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING C. I. BASIC RED 9 MONOHYDROCHLORIDE FOR TWO YEARS

Skin: Hyperkeratosis, basal cell hyperplasia, necrosis, and inflammation occurred at increased incidences in high dose male rats (Table 11). These lesions were not seen in dosed female rats.

Squamous cell carcinomas, squamous cell papillomas or carcinomas (combined), trichoepitheliomas, and sebaceous adenomas in male rats occurred with significant positive trends, and the incidences in the high dose group were significantly greater than those in the controls (Table 12). These neoplasms were not observed at significantly increased incidences in female rats.

Many of the male rats had multiple masses on the skin. These began to appear after about 13 months of exposure and were noted commonly on the sides of the animal. Squamous cell carcinomas were often well differentiated and comprised nests or sheets of epithelial cells in various stages of differentiation. Some had invaded the dermis. Keratin pearls were commonly found in the squamous cell. Carcinomas and keratohyalin granules were present in some cells. One squamous cell carcinoma metastasized to the lung in a high dose male rat. The sebaceous adenomas contained cells with a foamy cytoplasm and a centrally or eccentrically located nucleus. Trichoepitheliomas were characterized by nests or sheets of cells circumscribed by delicate or dense fibrovascular stroma. The cells were oval or polygonal with eosinophilic cytoplasm; the nuclei had coarse or granular cytoplasm. Some of the numerous small keratinous cores in the parenchyma were lined by concentric rings of squamous epithelial cells suggesting differentiation toward formation of hair.

TABLE 11. INCIDENCES OF NEOPLASTIC AND NONNEOPLASTIC SKIN LESIONS IN RATS IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Lesion	Concentration (ppm)			Concentration (ppm)		
	Control	1,000	2,000	Control	500	1,000
	Male (a)			Female (a)		
Inflammation	2	1	8	1	0	0
Necrosis	0	0	6	0	0	0
Hyperkeratosis	2	2	10	0	0	0
Keratoacanthoma	0	1	2	0	0	0
Sebaceous adenoma	0	0	5	0	0	0
Trichoepithelioma	0	0	7	0	0	0
Basal cell hyperplasia	0	0	5	0	0	0
Basal cell carcinoma	1	0	4	1	0	1
Squamous cell papilloma	2	1	4	0	0	0
Squamous cell carcinoma	0	1	10	0	0	1

(a) Fifty animals were examined in each group.

TABLE 12. ANALYSIS OF SKIN TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (a)

	Control	1,000 ppm (b)	2,000 ppm (b)
Squamous Cell Papilloma			
Overall Rates	2/50 (4%)	1/50 (2%)	4/50 (8%)
Squamous Cell Carcinoma			
Overall Rates	0/50 (0%)	1/50 (2%)	10/50 (20%)
Adjusted Rates	0.0%	3.4%	85.4%
Terminal Rates	0/36 (0%)	1/29 (3%)	0/0
Life Table Tests	P<0.001	P=0.457	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.500	P<0.001
Squamous Cell Papilloma or Carcinoma (c)			
Overall Rates	2/50 (4%)	2/50 (4%)	14/50 (28%)
Adjusted Rates	5.6%	6.9%	89.6%
Terminal Rates	2/36 (6%)	2/29 (7%)	0/0
Life Table Tests	P<0.001	P=0.615	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.691	P<0.001
Trichoepithelioma (d)			
Overall Rates	0/50 (0%)	0/50 (0%)	7/50 (14%)
Adjusted Rates	0.0%	0.0%	71.1%
Terminal Rates	0/36 (0%)	0/29 (0%)	0/0
Life Table Tests	P<0.001	(e)	P<0.001
Cochran-Armitage Trend Test	P=0.001		
Fisher Exact Tests		(e)	P=0.006
Sebaceous Adenoma (f)			
Overall Rates	0/50 (0%)	0/50 (0%)	5/50 (10%)
Adjusted Rates	0.0%	0.0%	27.3%
Terminal Rates	0/36 (0%)	0/29 (0%)	0/0
Life Table Tests	P<0.001	(e)	P=0.001
Cochran-Armitage Trend Test	P=0.006		
Fisher Exact Tests		(e)	P=0.028

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E.
(b) The estimated dose in milligrams per kilogram per day is given in Chapter III (Body Weights and Clinical Signs) and in Appendix L.
(c) Historical incidence at testing laboratory (mean ± SD): 17/699 (2% ± 1%); historical incidence in NTP studies: 44/2,372 (2% ± 2%)
(d) Historical incidence at testing laboratory (mean ± SD): 1/699 (0.1% ± 0.5%); historical incidence in NTP studies: 4/2,372 (0.2% ± 0.6%)
(e) No P value is reported because no tumors were observed in the control and 1,000-ppm groups.
(f) Historical incidence at testing laboratory (mean ± SD): 1/699 (0.1% ± 0.5%); historical incidence in NTP studies: 3/2,372 (0.1% ± 0.4%)

Subcutaneous Tissue: Fibromas occurred in male and female rats with significant positive trends, and the incidences in the dosed groups were significantly greater than those in the controls (Table 13). The fibromas were circumscribed, were either single or multiple, and varied in size. Fusiform cells occurred in palisades or whorls, and the fibromas contained mature collagen. A review of the gross descriptions revealed that none of these tumors was in the mammary gland area.

TABLE 13. ANALYSIS OF SUBCUTANEOUS TISSUE TUMORS IN RATS IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	Low Dose	High Dose
MALE		**1,000 ppm**	**2,000 ppm**
Fibroma			
Overall Rates	2/50 (4%)	20/50 (40%)	16/50 (32%)
Adjusted Rates	5.6%	47.8%	100.0%
Terminal Rates	2/36 (6%)	9/29 (31%)	0/0
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
Fibrosarcoma or Sarcoma			
Overall Rates	4/50 (8%)	5/50 (10%)	4/50 (8%)
Fibroma or Fibrosarcoma (a)			
Overall Rates	3/50 (6%)	22/50 (44%)	16/50 (32%)
Adjusted Rates	7.8%	53.0%	100.0%
Terminal Rates	2/36 (6%)	11/29 (38%)	0/0
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P=0.003		
Fisher Exact Tests		P<0.001	P<0.001
Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates	6/50 (12%)	24/50 (48%)	19/50 (38%)
Adjusted Rates	14.1%	55.5%	100.0%
Terminal Rates	3/36 (8%)	11/29 (38%)	0/0
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P=0.004		
Fisher Exact Tests		P<0.001	P=0.002
FEMALE		**500 ppm**	**1,000 ppm**
Fibroma			
Overall Rates	0/50 (0%)	15/50 (30%)	10/50 (20%)
Adjusted Rates	0.0%	37.8%	40.3%
Terminal Rates	0/37 (0%)	11/35 (31%)	3/14 (21%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P=0.005		
Fisher Exact Tests		P<0.001	P<0.001
Fibrosarcoma			
Overall Rates	0/50 (0%)	2/50 (4%)	0/50 (0%)
Fibroma or Fibrosarcoma (b)			
Overall Rates	0/50 (0%)	16/50 (32%)	10/50 (20%)
Adjusted Rates	0.0%	39.5%	40.3%
Terminal Rates	0/37 (0%)	11/35 (31%)	3/14 (21%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P=0.006		
Fisher Exact Tests		P<0.001	P<0.001
Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates	1/50 (2%)	17/50 (34%)	12/50 (24%)
Adjusted Rates	2.7%	40.9%	47.2%
Terminal Rates	1/37 (3%)	11/35 (31%)	3/14 (21%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P=0.004		
Fisher Exact Tests		P<0.001	P<0.001

(a) Historical incidence at testing laboratory (mean ± SD): 29/699 (4% ± 4%); historical incidence in NTP studies: 124/2,372 (5% ± 3%)
(b) Historical incidence at testing laboratory (mean ± SD): 15/747 (2% ± 2%); historical incidence in NTP studies: 42/2,422 (2% ± 2%)

Mammary Gland: Fibroadenomas and adenomas, fibroadenomas, or adenocarcinomas (combined) in female rats occurred with significant positive trends, and the incidence of fibroadenomas in the high dose group was significantly greater than in the controls (Table 14).

TABLE 14. ANALYSIS OF MAMMARY GLAND TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	500 ppm	1,000 ppm
Fibroadenoma			
Overall Rates	22/50 (44%)	31/50 (62%)	29/50 (58%)
Adjusted Rates	49.7%	73.4%	96.2%
Terminal Rates	15/37 (41%)	24/35 (69%)	13/14 (93%)
Life Table Tests	P<0.001	P=0.061	P<0.001
Cochran-Armitage Trend Test	P=0.096		
Fisher Exact Tests		P=0.054	P=0.115
Adenoma or Fibroadenoma (a)			
Overall Rates	22/50 (44%)	31/50 (62%)	29/50 (58%)
Adjusted Rates	49.7%	73.4%	96.2%
Terminal Rates	15/37 (41%)	24/35 (69%)	13/14 (93%)
Life Table Tests	P<0.001	P=0.061	P<0.001
Cochran-Armitage Trend Test	P=0.096		
Fisher Exact Tests		P=0.054	P=0.115
Adenocarcinoma			
Overall Rates	2/50 (4%)	2/50 (4%)	5/50 (10%)
Adenoma, Fibroadenoma, or Adenocarcinoma			
Overall Rates	23/50 (46%)	32/50 (64%)	32/50 (64%)
Adjusted Rates	52.0%	75.8%	96.6%
Terminal Rates	16/37 (43%)	25/35 (71%)	13/14 (93%)
Life Table Tests	P<0.001	P=0.059	P<0.001
Cochran-Armitage Trend Test	P=0.043		
Fisher Exact Tests		P=0.054	P=0.054

(a) Historical incidence at testing laboratory (mean ± SD): 226/747 (30% ± 9%); historical incidence in NTP studies: 549/2,422 (23% ± 10%)

Thyroid Gland: Follicular cell hyperplasia was observed in 16/44 high dose males and 2/48 low dose females but not in other groups of male or female rats (Table 15). Follicular cysts were observed in 3/46 (7%) low dose males, 3/44 (7%) high dose males, 3/48 (6%) low dose females, and 2/50 (4%) high dose females but in none of the controls. A large or distended follicle with eosinic or pale colloid and lined by cuboidal epithelial cells was considered a follicular cyst. Features of hyperplasia included diffuse follicular enlargement or papillary ingrowth of the epithelium resulting in follicles of various sizes. The cells were either columnar or cuboidal.

Follicular cell adenomas in males and females, follicular cell carcinomas in males, and follicular cell adenomas or carcinomas (combined) in males and females occurred with statistically significant positive trends. The incidences of follicular cell adenomas and follicular cell carcinomas in high dose males, follicular cell adenomas or carcinomas (combined) in high dose males and females, and follicular cell carcinomas in low dose males were significantly greater than those in the controls (Table 16).

Follicular cell neoplasms were vascularized and contained a prominent fibrous capsule. Follicular cell adenomas involved part or all of a lobe and compressed the adjacent tissue. Both macrofollicular and microfollicular types were recognized. The cytoplasm of the cells stained bright red, and nuclei were hyperchromatic. A follicular papillary pattern of the cells and infiltration of the cells into the capsule or blood vessels were features of a follicular cell carcinoma. Areas of necrosis, hemorrhage, pigment, and mineralization were common in carcinomas. A follicular cell carcinoma metastasized to the lung in one low dose male rat.

TABLE 15. NUMBERS OF RATS WITH NONNEOPLASTIC LESIONS OF THE THYROID GLAND IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	Low Dose	High Dose
MALE		**1,000 ppm**	**2,000 ppm**
Number examined microscopically	49	46	44
Mineralization	0	0	1 (2%)
Follicular cyst	0	3 (7%)	3 (7%)
Inflammation	0	0	1 (2%)
Fibrosis	0	0	1 (2%)
Necrosis	0	0	1 (2%)
Hyperplasia, C-cell	2 (4%)	2 (4%)	0
Hyperplasia, follicular cell	0	0	16 (36%)
Metaplasia, squamous	0	0	1 (2%)
FEMALE		**500 ppm**	**1,000 ppm**
Number examined microscopically	47	48	50
Follicular cyst	0	3 (6%)	2 (4%)
Necrosis	0	1 (2%)	0
Hyperplasia, C-cell	7 (15%)	4 (8%)	0
Hyperplasia, follicular cell	0	2 (4%)	0

TABLE 16. ANALYSIS OF THYROID GLAND LESIONS IN RATS IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	Low Dose	High Dose
MALE		**1,000 ppm**	**2,000 ppm**
Follicular Cell Hyperplasia			
Overall Rates	0/49 (0%)	0/46 (0%)	16/44 (36%)
Follicular Cell Adenoma			
Overall Rates	0/49 (0%)	0/46 (0%)	9/44 (20%)
Adjusted Rates	0.0%	0.0%	78.4%
Terminal Rates	0/36 (0%)	0/27 (0%)	0/0
Life Table Tests	P<0.001	(a)	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		(a)	P<0.001
Follicular Cell Carcinoma			
Overall Rates	0/49 (0%)	5/46 (11%)	18/44 (41%)
Adjusted Rates	0.0%	15.9%	81.6%
Terminal Rates	0/36 (0%)	3/27 (11%)	0/0
Life Table Tests	P<0.001	P=0.020	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.024	P<0.001
Follicular Cell Adenoma or Carcinoma (b)			
Overall Rates	0/49 (0%)	5/46 (11%)	25/44 (57%)
Adjusted Rates	0.0%	15.9%	91.4%
Terminal Rates	0/36 (0%)	3/27 (11%)	0/0
Life Table Tests	P<0.001	P=0.020	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.024	P<0.001
FEMALE		**500 ppm**	**1,000 ppm**
Follicular Cell Hyperplasia			
Overall Rates	0/47 (0%)	2/48 (4%)	0/50 (0%)
Follicular Cell Adenoma			
Overall Rates	0/47 (0%)	0/48 (0%)	4/50 (8%)
Adjusted Rates	0.0%	0.0%	20.4%
Terminal Rates	0/37 (0%)	0/33 (0%)	1/14 (7%)
Life Table Tests	P=0.002	(c)	P=0.009
Cochran-Armitage Trend Test	P=0.017		
Fisher Exact Tests		(c)	P=0.066
Follicular Cell Carcinoma			
Overall Rates	0/47 (0%)	2/48 (4%)	2/50 (4%)
Follicular Cell Adenoma or Carcinoma (d)			
Overall Rates	0/47 (0%)	2/48 (4%)	6/50 (12%)
Adjusted Rates	0.0%	5.3%	29.2%
Terminal Rates	0/37 (0%)	1/33 (3%)	2/14 (14%)
Life Table Tests	P<0.001	P=0.232	P<0.001
Cochran-Armitage Trend Test	P=0.009		
Fisher Exact Tests		P=0.253	P=0.016

(a) No P value is reported because no tumors were observed in 1,000-ppm and control groups.
(b) Historical incidence at testing laboratory (mean ± SD): 4/664 (0.6% ± 1%); historical incidence in NTP studies: 38/2,282 (2% ± 2%)
(c) No P value is reported because no tumors were observed in the 500-ppm and control groups.
(d) Historical incidence at testing laboratory (mean ± SD): 3/724 (0.4% ± 1%); historical incidence in NTP studies: 15/2,317 (0.6% ± 1%)

Zymbal Gland: Necrosis and hyperkeratosis of the Zymbal gland were observed in high dose male and dosed female rats but not in the controls (necrosis: male--control, 0/50; low dose, 1/50, 2%; high dose, 6/50, 12%; female--control, 0/50; low dose, 1/50, 2%; high dose, 5/50, 10%; hyperkeratosis: male--control, 0/50; low dose, 1/50, 2%; high dose, 8/50, 16%; female--control, 0/50; low dose, 2/50, 4%; high dose, 3/50, 6%).

Zymbal gland carcinomas in male and female rats occurred with significant positive trends, and the incidences in the high dose groups were significantly greater than those in the controls (Table 17). These carcinomas were generally large. A fairly uniform population of cells occurred in sheets; many cells had a foamy cytoplasm. Nuclei were vesicular or had coarse chromatin. Hyperkeratosis and necrosis were common in many of these tumors.

TABLE 17. ANALYSIS OF ZYMBAL GLAND TUMORS IN RATS IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	Low Dose	High Dose
MALE		**1,000 ppm**	**2,000 ppm**
Carcinoma (a)			
Overall Rates	1/50 (2%)	1/50 (2%)	13/50 (26%)
Adjusted Rates	2.4%	3.4%	80.5%
Terminal Rates	0/36 (0%)	1/29 (3%)	0/0
Life Table Tests	P<0.001	P=0.715	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.753	P<0.001
FEMALE		**500 ppm**	**1,000 ppm**
Carcinoma (b)			
Overall Rates	0/50 (0%)	2/50 (4%)	7/50 (14%)
Adjusted Rates	0.0%	4.0%	24.1%
Terminal Rates	0/37 (0%)	0/35 (0%)	1/14 (7%)
Life Table Tests	P<0.001	P=0.261	P=0.002
Cochran-Armitage Trend Test	P=0.003		
Fisher Exact Tests		P=0.247	P=0.006

(a) Historical incidence at testing laboratory (mean ± SD): 3/699 (0.4% ± 1%); historical incidence in NTP studies: 11/2,372 (0.5% ± 1%)
(b) Historical incidence at testing laboratory (mean ± SD): 2/747 (0.3% ± 1%); historical incidence in NTP studies: 6/2,422 (0.2% ± 1%)

Liver: Neoplastic nodules in males, hepatocellular carcinomas in males, and neoplastic nodules or hepatocellular carcinomas (combined) in males and females occurred with significant positive trends (Table 18). The incidences of neoplastic nodules in low dose males, hepatocellular carcinomas in high dose males, and neoplastic nodules or carcinomas (combined) in dosed males were significantly greater than those in the controls. Neoplastic nodules compressed the adjacent liver tissue. Multiple nodules were found in some rats. Cells in the nodules were generally larger than normal hepatocytes, and the cytoplasmic staining was varied. The nucleus in many cells had a stippled chromatin and a large nucleolus. Hepatocellular carcinomas involved part or all of a lobe of the liver. In some tumors, dense fibrovascular stroma had dissected the tumor parenchyma into nodules of varying sizes. The cells were arranged in sheets or in trabecular or acinar patterns. The cytoplasm stained eosinophilic or basophilic or was vacuolated. A few nucleoli had inclusions. The hepatocellular carcinomas metastasized to the lung in one low dose and two high dose males.

The incidence of necrosis (primarily focal or ischemic) of the liver was increased in high dose male rats (control, 2/50, 4%; low dose, 4/50, 8%; high dose, 20/50, 40%). One bile duct adenoma and two bile duct carcinomas were found in high dose male rats. The incidence of bile duct adenomas or carcinomas (combined) in male rats was significant by trend tests. These lesions may be compound related, since they are very uncommon in NTP historical controls (1/2,358). However, each lesion occurred in a liver that already had a hepatocellular carcinoma. Further, each lesion was distinct and different morphologically from the others. Finally, it was difficult to determine whether one lesion represented a distinct entity or was bile duct proliferation as part of the hepatocellular carcinoma.

Urinary System: Two uncommon tumors were observed in low dose female rats. One rat had a renal tubular cell adenoma, and a second had a transitional cell carcinoma of the urinary bladder. Two transitional cell urinary bladder tumors have been observed in 728 female controls at the testing laboratory, and no renal tubular cell tumors have been observed in 742 female controls. The incidence of renal tubular cell tumors in NTP studies is 4/2,411 (0.2%) and that of urinary bladder transitional cell tumors is 4/2,422 (0.2%).

TABLE 18. ANALYSIS OF LIVER TUMORS IN RATS IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	Low Dose	High Dose
MALE		**1,000 ppm**	**2,000 ppm**
Neoplastic Nodule			
Overall Rates	5/50 (10%)	14/50 (28%)	6/50 (12%)
Adjusted Rates	13.9%	46.3%	38.9%
Terminal Rates	5/36 (14%)	13/29 (45%)	0/0
Life Table Tests	P<0.001	P=0.004	P<0.001
Cochran-Armitage Trend Test	P=0.447		
Fisher Exact Tests		P=0.020	P=0.500
Hepatocellular Carcinoma			
Overall Rates	0/50 (0%)	2/50 (4%)	8/50 (16%)
Adjusted Rates	0.0%	6.9%	57.4%
Terminal Rates	0/36 (0%)	2/29 (7%)	0/0
Life Table Tests	P<0.001	P=0.192	P<0.001
Cochran-Armitage Trend Test	P=0.001		
Fisher Exact Tests		P=0.247	P=0.003
Neoplastic Nodule or Hepatocellular Carcinoma (a)			
Overall Rates	5/50 (10%)	15/50 (30%)	14/50 (28%)
Adjusted Rates	13.9%	49.6%	74.0%
Terminal Rates	5/36 (14%)	14/29 (48%)	0/0
Life Table Tests	P<0.001	P=0.002	P<0.001
Cochran-Armitage Trend Test	P=0.021		
Fisher Exact Tests		P=0.011	P=0.020
Bile Duct Adenoma or Carcinoma (b)			
Overall Rates	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted Rates	0.0%	0.0%	25.5%
Terminal Rates	0/36 (0%)	0/29 (0%)	0/0
Life Table Tests	P=0.002	(c)	P=0.005
Cochran-Armitage Trend Test	P=0.037		
Fisher Exact Tests		(c)	P=0.121
FEMALE		**500 ppm**	**1,000 ppm**
Neoplastic Nodule			
Overall Rates	1/50 (2%)	4/50 (8%)	3/50 (6%)
Adjusted Rates	2.7%	11.0%	8.4%
Terminal Rates	1/37 (3%)	3/35 (9%)	0/14 (0%)
Life Table Tests	P=0.073	P=0.170	P=0.174
Cochran-Armitage Trend Test	P=0.252		
Fisher Exact Tests		P=0.181	P=0.309
Hepatocellular Carcinoma			
Overall Rates	0/50 (0%)	0/50 (0%)	1/50 (2%)
Neoplastic Nodule or Hepatocellular Carcinoma (d)			
Overall Rates	1/50 (2%)	4/50 (8%)	4/50 (8%)
Adjusted Rates	2.7%	11.0%	14.9%
Terminal Rates	1/37 (3%)	3/35 (9%)	1/14 (7%)
Life Table Tests	P=0.025	P=0.170	P=0.062
Cochran-Armitage Trend Test	P=0.146		
Fisher Exact Tests		P=0.181	P=0.181

(a) Historical incidence at testing laboratory (mean ± SD): 25/693 (4% ± 4%); historical incidence in NTP studies: 110/2,358 (5% ± 5%)
(b) Historical incidence at testing laboratory (mean): 1/693 (0.1%); historical incidence in NTP studies: 1/2,358 (<0.1%)
(c) No P value is reported because no tumors were observed in the 1,000-ppm and control groups.
(d) Historical incidence at testing laboratory (mean ± SD): 26/741 (4% ± 3%); historical incidence in NTP studies: 89/2,408 (4% ± 5%)

Uterus: Endometrial stromal sarcomas alone occurred with a significant positive trend; however, the sarcomas appeared to originate in stromal polyps (Table 19). Therefore, the incidence of stromal sarcomas was combined with that of stromal polyps; the combined incidence was significantly greater than that in the controls by the life table test. The sarcomas were well vascularized in some rats and had a striking resemblance to vascular neoplasms. The cells were pleomorphic in size and shape. The fusiform cells were arranged in whorls or palisades; the large polygonal cells were arranged in sheets or nests. Cells with bizarre or multiple nuclei were numerous in some neoplasms. Adenocarcinomas of the uterus were observed in two high dose female rats.

TABLE 19. ANALYSIS OF UTERINE TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	500 ppm	1,000 ppm
Endometrial Stromal Polyp (a)			
Overall Rates	15/50 (30%)	14/50 (28%)	10/49 (20%)
Adjusted Rates	36.9%	35.3%	41.4%
Terminal Rates	12/37 (32%)	10/35 (29%)	4/14 (29%)
Life Table Tests	P=0.225	P=0.541N	P=0.266
Cochran-Armitage Trend Test	P=0.166N		
Fisher Exact Tests		P=0.500N	P=0.193
Endometrial Stromal Sarcoma (b)			
Overall Rates	1/50 (2%)	5/50 (10%)	6/49 (12%)
Adjusted Rates	2.7%	13.3%	22.6%
Terminal Rates	1/37 (3%)	4/35 (11%)	1/14 (7%)
Life Table Tests	P=0.004	P=0.099	P=0.010
Cochran-Armitage Trend Test	P=0.045		
Fisher Exact Tests		P=0.102	P=0.053
Endometrial Stromal Polyp or Sarcoma			
Overall Rates	16/50 (32%)	18/50 (36%)	16/49 (33%)
Adjusted Rates	394%	44.3%	56.2%
Terminal Rates	13/37 (35%)	13/35 (37%)	5/14 (36%)
Life Table Tests	P=0.014	P=0.379	P=0.024
Cochran-Armitage Trend Test	P=0.514		
Fisher Exact Tests		P=0.416	P=0.558

(a) Historical incidence at testing laboratory (mean ± SD): 154/733 (21% ± 8%); historical incidence in NTP studies: 429/2,370 (18% ± 8%)
(b) Historical incidence at testing laboratory (mean ± SD): 7/733 (1% ± 2%); historical incidence in NTP studies: 22/2,370 (0.9% ± 2%)

Lung: Alveolar/bronchiolar adenomas and alveolar/bronchiolar adenomas or carcinomas (combined) in dosed male rats occurred with significant positive trends, and the incidence of alveolar/bronchiolar adenomas or carcinomas (combined) in high dose male rats was increased over controls by the life table tests (Table 20).

Hematopoietic System: Leukemia in male rats occurred with a significant negative trend, and the incidences in the dosed groups were significantly lower (P=0.01) than those in the controls (control, 7/50, 14%; low dose, 1/50, 2%; high dose, 1/50, 2%). The incidence of leukemia in the high dose male rats may be lower than that of the controls because of decreased survival.

TABLE 20. ANALYSIS OF LUNG TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	1,000 ppm	2,000 ppm
Alveolar/Bronchiolar Adenoma			
Overall Rates	0/50 (0%)	3/50 (6%)	3/50 (6%)
Adjusted Rates	0.0%	8.9%	8.4%
Terminal Rates	0/36 (0%)	2/29 (7%)	0/0
Life Table Tests	P=0.008	P=0.101	P=0.076
Cochran-Armitage Trend Test	P=0.101		
Fisher Exact Tests		P=0.121	P=0.121
Alveolar/Bronchiolar Carcinoma			
Overall Rates	1/50 (2%)	0/50 (0%)	1/50 (2%)
Alveolar/Bronchiolar Adenoma or Carcinoma (a)			
Overall Rates	1/50 (2%)	3/50 (6%)	4/50 (8%)
Adjusted Rates	2.4%	8.9%	31.3%
Terminal Rates	0/36 (0%)	2/29 (7%)	0/0
Life Table Tests	P=0.004	P=0.258	P=0.017
Cochran-Armitage Trend Test	P=0.133		
Fisher Exact Tests		P=0.309	P=0.181

(a) Historical incidence at testing laboratory (mean ± SD): 13/696 (2% ± 2%); historical incidence in NTP studies: 57/2,357 (2% ± 2%)

THIRTEEN-WEEK STUDIES

One male that received 2,000 ppm C.I. Basic Red 9 monohydrochloride died on day 5 (Table 21). This death was probably not compound related, since no deaths occurred at 4,000 ppm during 13 weeks of exposure. One female that received 2,000 ppm died accidentally during week 12. Final mean body weights relative to those of the controls were at least 10% lower for male mice that received 4,000 ppm and for female mice that received 1,000 ppm or more. No compound-related clinical signs of toxicity or histopathologic effects were observed. Mice that received 4,000 ppm had a higher rate of feed consumption than did the other groups.

Dose Selection Rationale: Because of weight gain depression in males at 4,000 ppm and in females at 2,000 ppm, doses selected for mice for the 2-year studies were 500 or 1,000 ppm C.I. Basic Red 9 monohydrochloride in feed.

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of dosed mice of each sex were lower than those of the controls throughout the studies (Table 22 and Figure 3). The average daily feed consumption by low dose and high dose male mice was 97% and 92% that of the controls and by low dose and high dose female mice, 120% and 155% that of the controls (Appendix M, Tables M3 and M4). Feed consumption measurements are not corrected for scatter. The average daily doses of C.I. Basic Red 9 monohydrochloride were approximately 196 and 379 mg/kg body weight for low dose and high dose male mice and 149 and 407 mg/kg body weight for low dose and high dose female mice.

TABLE 21. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Concentration (ppm)	Survival (a)	Mean Body Weight (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 4	Week 12
MALE							
0	10/10	22.7 ± 0.6	33.5 ± 0.9	+ 10.8 ± 0.5	--	282	339
250	10/10	22.3 ± 0.5	32.0 ± 1.1	+ 9.7 ± 0.8	95.5	270	355
500	10/10	21.3 ± 0.7	30.2 ± 1.1	+ 8.9 ± 0.9	90.1	300	343
1,000	10/10	21.7 ± 0.5	31.3 ± 0.8	+ 9.6 ± 0.4	93.4	222	337
2,000	(e) 9/10	21.6 ± 0.5	30.5 ± 0.6	+ 8.9 ± 0.3	91.0	321	377
4,000	10/10	21.7 ± 0.4	28.8 ± 0.4	+ 7.1 ± 0.4	86.0	413	514
FEMALE							
0	10/10	18.6 ± 0.3	29.5 ± 0.9	+10.9 ± 0.8	--	248	311
250	10/10	18.5 ± 0.2	27.8 ± 0.8	+ 9.3 ± 0.7	94.2	194	304
500	10/10	18.6 ± 0.3	27.1 ± 0.5	+ 8.5 ± 0.5	91.9	210	346
1,000	10/10	18.5 ± 0.3	26.5 ± 0.5	+ 8.0 ± 0.4	89.8	217	385
2,000	(f) 9/10	18.6 ± 0.3	25.3 ± 0.4	+ 6.6 ± 0.4	85.8	253	368
4,000	10/10	18.4 ± 0.3	23.5 ± 0.4	+ 5.1 ± 0.2	79.7	320	465

(a) Number surviving/number initially in the group
(b) Initial mean body weight ± standard error of the mean of all animals in the group. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean weight change of the survivors of the group ± standard error of the mean
(d) Grams of feed consumed per kilograms of body weight per day
(e) Week of death: 1
(f) Death judged to be accidental

TABLE 22. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Weeks on Study	Control		500 ppm			1,000 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE								
0	27.0	50	26.2	97	50	26.3	97	50
1	28.6	50	28.1	98	49	28.4	99	50
2	28.8	50	28.6	99	49	28.6	99	50
3	29.0	50	28.5	98	48	29.0	100	50
4	29.9	50	29.0	97	48	29.7	99	50
5	29.9	50	29.6	99	48	29.9	100	50
6	30.9	50	29.9	97	48	30.5	99	50
7	31.5	50	30.2	96	48	31.1	99	50
8	33.0	50	31.5	95	48	31.9	97	50
9	31.9	50	30.8	97	48	31.2	98	50
10	33.3	50	32.0	96	48	32.6	98	50
11	34.3	50	32.9	96	48	32.8	96	50
12	35.5	50	33.3	94	48	33.8	95	50
16	37.0	50	34.5	93	48	35.0	95	49
20	38.7	50	35.8	93	48	36.2	94	49
24	37.8	50	35.4	94	48	35.1	93	49
28	39.0	50	37.1	95	48	36.5	94	49
32	40.6	50	37.6	93	48	37.2	92	49
36	42.6	50	38.6	91	48	38.0	89	49
40	43.9	50	40.4	92	47	39.7	90	48
44	45.1	50	41.1	91	47	40.4	90	48
48	45.4	50	41.2	91	47	40.5	89	48
52	46.4	50	42.1	91	47	40.6	88	47
64	47.3	50	43.2	91	46	41.8	88	47
68	48.9	49	42.8	88	46	41.4	85	45
72	46.6	49	41.9	90	46	40.1	86	45
76	47.7	48	41.8	88	43	40.4	85	44
80	47.5	48	41.9	88	40	39.7	84	43
84	47.0	46	40.9	87	40	38.9	83	42
88	46.6	46	40.8	88	38	39.0	84	40
92	45.9	43	38.8	85	37	37.4	81	39
96	45.4	43	39.1	86	35	37.2	82	39
100	45.1	43	39.5	88	33	36.7	81	37
104	43.4	42	37.9	87	32	34.7	80	36
FEMALE								
0	20.5	50	20.4	100	50	20.8	101	50
1	21.5	50	21.3	99	50	21.5	100	50
2	22.7	50	22.2	98	50	23.1	102	50
3	23.6	50	24.0	102	50	23.2	98	50
4	26.8	50	23.3	87	50	23.6	88	50
5	25.1	50	24.1	96	50	24.2	96	50
6	25.1	50	24.6	98	50	24.9	99	50
7	25.4	50	24.9	98	50	24.5	96	50
8	25.7	50	25.3	98	50	25.4	99	50
9	26.1	50	25.8	99	50	25.8	99	50
10	27.3	50	25.8	95	50	26.3	96	50
11	26.9	50	25.9	96	50	26.4	98	50
12	28.2	50	27.0	96	50	27.2	96	50
16	30.2	50	28.2	93	50	28.1	93	50
20	30.5	50	28.4	93	50	28.5	93	50
24	32.8	50	30.6	93	50	30.5	93	50
28	34.4	50	31.0	90	50	29.4	85	50
32	36.8	50	31.8	86	50	31.0	84	49
36	39.1	50	32.8	84	49	31.8	81	49
40	40.3	50	34.0	84	48	32.5	81	49
44	42.5	50	36.3	85	48	34.1	80	49
48	44.5	49	36.4	82	48	34.2	77	49
52	46.2	49	37.0	80	46	34.9	76	49
56	46.4	49	37.3	80	45	35.4	76	48
60	47.6	49	37.5	79	44	35.0	74	48
64	47.5	49	37.0	78	41	34.0	72	46
68	48.1	49	37.2	77	40	33.3	69	41
72	49.0	48	36.4	74	40	32.6	67	40
76	50.1	47	36.5	73	39	32.5	65	39
80	52.3	47	37.2	71	37	32.0	61	36
84	53.5	44	37.4	70	31	31.9	60	32
88	52.5	42	35.9	68	29	30.4	58	25
92	49.9	40	34.7	70	24	31.2	63	21
94	--	--	35.5	--	21	31.0	--	18
96	50.9	36	35.2	69	21	30.5	60	12
98	--	--	34.4	--	20	30.3	--	10
100	50.7	34	34.2	67	17	31.4	62	8
102	--	--	32.9	--	16	31.3	--	6
104	48.6	31	31.9	66	13	30.8	63	6

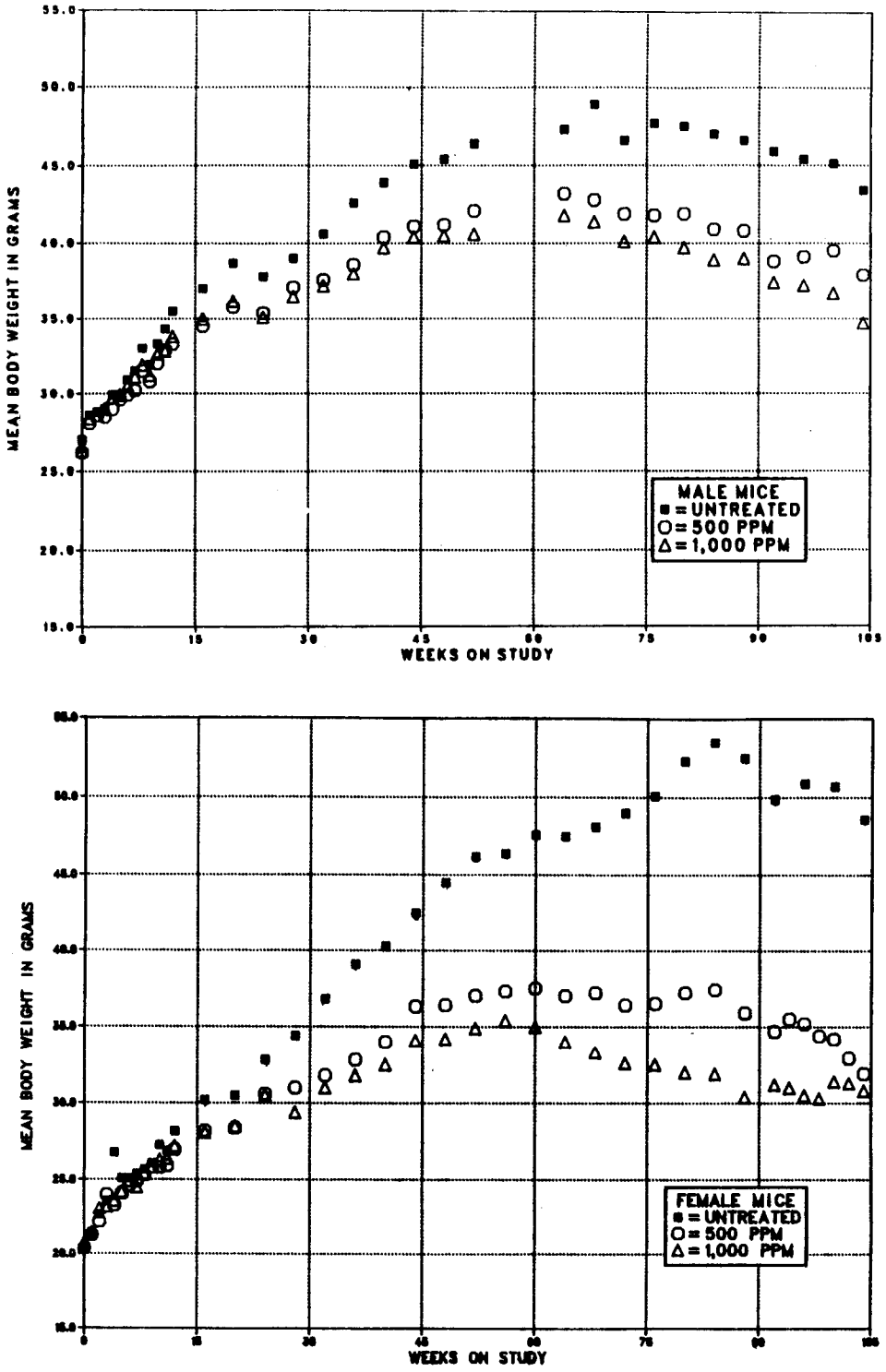


FIGURE 3. GROWTH CURVES FOR MICE FED DIETS CONTAINING C. I. BASIC RED 9 MONOHYDROCHLORIDE FOR TWO YEARS

Survival

Estimates of the probabilities of survival of male and female mice fed diets containing C.I. Basic Red 9 monohydrochloride at the concentrations used in these studies and those of the controls are shown in the Kaplan and Meier curves in Figure 4. The survival of the low dose group of male mice was significantly lower than that of the control group. In female mice, the survival of both dosed groups was significantly lower than that of the control group (Table 23).

In the initial study, fighting caused high mortality in the group-housed male mice. The male mice were restarted approximately 11 months after the female study and were individually housed. The doses (0, 500, 1,000 ppm) used in the original study were the same as those used in the restarted study of male mice.

Pathology and Statistical Analyses of Results

This section describes significant or noteworthy changes in the incidence of mice with neoplastic or nonneoplastic lesions of the lung, liver, adrenal gland, harderian gland, and hematopoietic system. Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); Appendix B (Tables B3 and B4) also gives the survival and tumor status for individual male and female mice. Findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2). Appendix E (Tables E3 and E4) contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes). Historical incidences of tumors in control animals are listed in Appendix F.

The statistical analyses and interpretation of the tumor incidence data for low dose and high dose female mice were complicated by the marked reduction in survival in this group when compared with that of the controls. In this situation, the incidental tumor test has relatively little sensitivity; hence, results of this test for female mice were not given major emphasis but, for completeness, are included in Appendix E. Instead, in this section, the results of unadjusted analyses (Fisher exact test and Cochran-Armitage test) and life table analyses are presented. A positive effect by both life table and unadjusted analyses was considered evidence that an increase in tumor incidence was related to chemical administration, except when neoplasms are clearly recognized as the cause of death (life table analysis would be appropriate in this instance).

TABLE 23. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	500 ppm	1,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	8	18	14
Killed at termination	42	31	35
Died during termination period	0	1	1
Survival P values (c)	0.192	0.032	0.190
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	19	38	44
Killed at termination	31	12	6
Survival P values (c)	<0.001	<0.001	<0.001

(a) Terminal kill period: male--weeks 104-105; female--week 105
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.

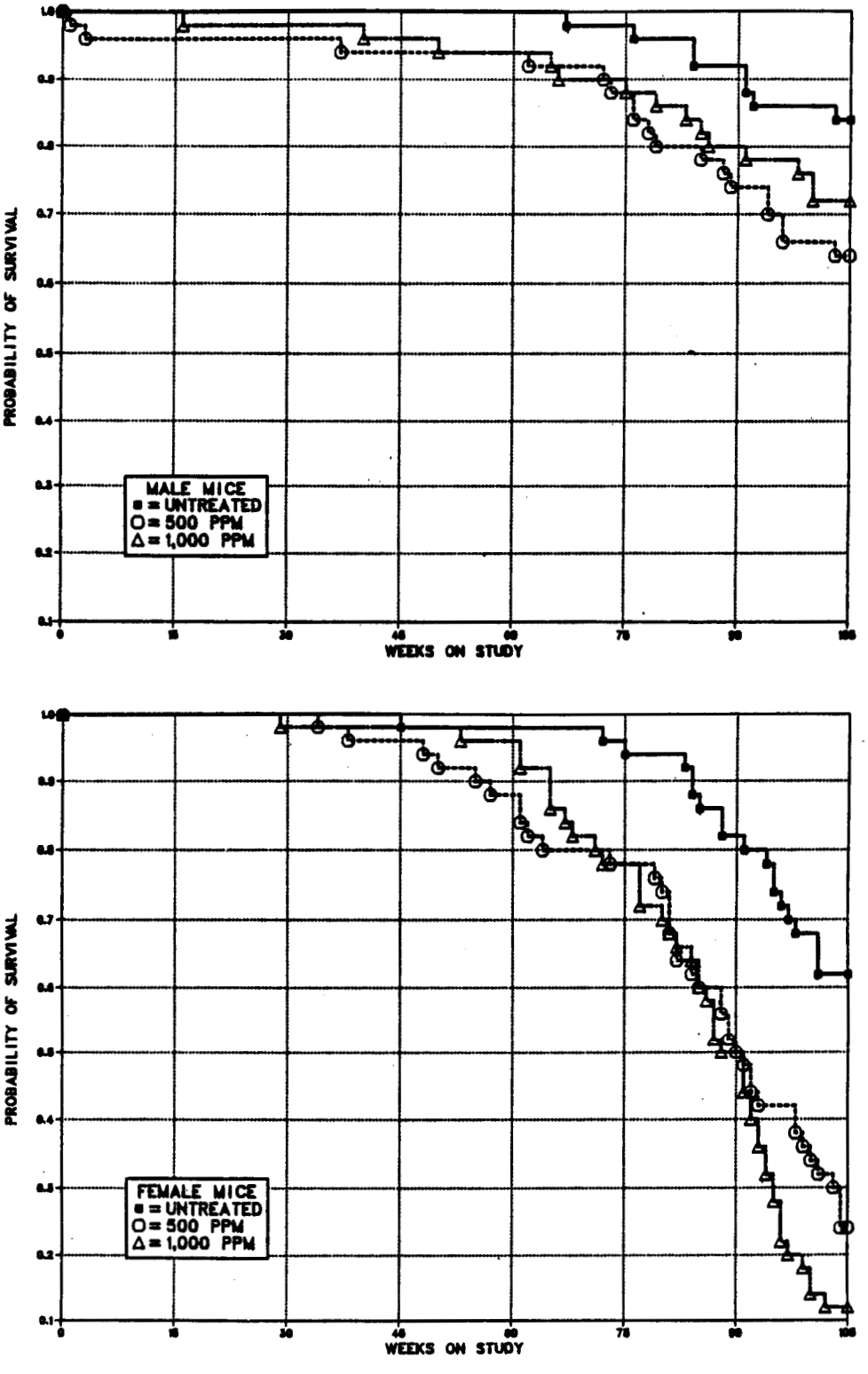


FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING C. I. BASIC RED 9 MONOHYDROCHLORIDE FOR TWO YEARS

Lung: Alveolar/bronchiolar adenomas and alveolar/bronchiolar adenomas or carcinomas (combined) in female mice occurred with significant positive trends (Table 24). The incidence of adenomas or carcinomas (combined) in high dose group was significantly greater than that in concurrent controls. The historical incidence for adenomas or carcinomas is 10% ± 5% at the testing laboratory and 7% ± 4% in NTP studies.

Liver: Hepatocellular carcinomas and hepatocellular adenomas or carcinomas (combined) in male and female mice occurred with statistically significant positive trends (Table 25). The incidences of hepatocellular carcinomas and hepatocellular adenomas or carcinomas (combined) in dosed males and females and of hepatocellular adenomas in low dose female mice were significantly greater than those in the controls. Hepatocellular adenomas compressed the adjacent parenchyma. The cells of the adenomas were large, the cytoplasm stained eosinophilic, and the nuclei had coarse or stippled chromatin. The hepatocellular carcinomas involved part or all of a lobe of the liver. Trabecular, acinar, or pseudoglandular patterns were common.

Cytoplasmic staining in the carcinomas varied, and eosinophilic globules were present in some cells. An occasional nucleus had inclusions. Sinusoids were dilated and/or congested. Areas of necrosis, hemorrhage, and mineralization were common in large tumors.

Hepatocellular carcinomas metastasized to the lung in 5 low dose males, 12 high dose males, 3 low dose females, and 13 high dose females; to the kidney in 1 high dose male; and to the lymph node in 1 high dose female. Necrosis was observed at increased incidences in dosed female mice (control, 10/49, 20%; low dose, 15/50, 30%; high dose, 26/49, 52%).

TABLE 24. ANALYSIS OF LUNG LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (a)

	Control	500 ppm (b)	1,000 ppm (b)
Epithelial Hyperplasia			
Overall Rates	1/50 (2%)	0/49 (0%)	0/47 (0%)
Alveolar/Bronchiolar Adenoma			
Overall Rates	0/50 (0%)	2/49 (4%)	4/47 (9%)
Adjusted Rates	0.0%	11.4%	25.2%
Terminal Rates	0/31 (0%)	1/12 (8%)	1/6 (17%)
Life Table Tests	P=0.004	P=0.112	P=0.011
Cochran-Armitage Trend Test	P=0.032		
Fisher Exact Tests		P=0.242	P=0.051
Alveolar/Bronchiolar Carcinoma			
Overall Rates	0/50 (0%)	0/49 (0%)	1/47 (2%)
Alveolar/Bronchiolar Adenoma or Carcinoma (c)			
Overall Rates	0/50 (0%)	2/49 (4%)	5/47 (11%)
Adjusted Rates	0.0%	11.4%	27.6%
Terminal Rates	0/31 (0%)	1/12 (8%)	1/6 (17%)
Life Table Tests	P=0.002	P=0.112	P=0.005
Cochran-Armitage Trend Test	P=0.014		
Fisher Exact Tests		P=0.242	P=0.024

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes).
(b) The estimated dose in milligrams per kilogram per day is given in Chapter III (Body Weights and Clinical Signs) and in Appendix L.
(c) Historical incidence at testing laboratory (mean ± SD): 73/745 (10% ± 5%); historical incidence in NTP studies: 179/2,439 (7% ± 4%)

TABLE 25. ANALYSIS OF LIVER TUMORS IN MICE IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	500 ppm	1,000 ppm
MALE			
Hepatocellular Adenoma			
Overall Rates	22/50 (44%)	21/50 (42%)	17/50 (34%)
Adjusted Rates	48.7%	55.9%	44.7%
Terminal Rates	19/42 (45%)	16/32 (50%)	15/36 (42%)
Life Table Tests	P=0.395N	P=0.260	P=0.413N
Incidental Tumor Tests	P=0.264N	P=0.532	P=0.239N
Cochran-Armitage Trend Test	P=0.179N		
Fisher Exact Tests		P=0.500N	P=0.206N
Hepatocellular Carcinoma			
Overall Rates	10/50 (20%)	20/50 (40%)	27/50 (54%)
Adjusted Rates	23.1%	49.0%	62.5%
Terminal Rates	9/42 (21%)	12/32 (38%)	20/36 (56%)
Life Table Tests	P<0.001	P=0.005	P<0.001
Incidental Tumor Tests	P<0.001	P=0.017	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.024	P<0.001
Hepatocellular Adenoma or Carcinoma (a)			
Overall Rates	29/50 (58%)	37/50 (74%)	41/50 (82%)
Adjusted Rates	62.8%	83.8%	91.1%
Terminal Rates	25/42 (60%)	25/32 (78%)	32/36 (89%)
Life Table Tests	P=0.001	P=0.004	P=0.001
Incidental Tumor Tests	P<0.001	P=0.035	P=0.002
Cochran-Armitage Trend Test	P=0.005		
Fisher Exact Tests		P=0.069	P=0.008
FEMALE			
Hepatocellular Adenoma			
Overall Rates	2/49 (4%)	18/50 (36%)	4/49 (8%)
Adjusted Rates	6.5%	73.8%	22.6%
Terminal Rates	2/31 (6%)	7/12 (58%)	1/6 (17%)
Life Table Tests	P=0.004	P<0.001	P=0.063
Cochran-Armitage Trend Test	P=0.341		
Fisher Exact Tests		P<0.001	P=0.339
Hepatocellular Carcinoma			
Overall Rates	3/49 (6%)	19/50 (38%)	37/49 (76%)
Adjusted Rates	9.2%	70.5%	97.1%
Terminal Rates	2/31 (6%)	6/12 (50%)	5/6 (83%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
Hepatocellular Adenoma or Carcinoma (b)			
Overall Rates	5/49 (10%)	35/50 (70%)	41/49 (84%)
Adjusted Rates	15.5%	96.9%	100.0%
Terminal Rates	4/31 (13%)	11/12 (92%)	6/6 (100%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001

(a) Historical incidence at testing laboratory (mean ± SD): 237/745 (32% ± 9%); historical incidence in NTP studies: 730/2,386 (31% ± 8%)
(b) Historical incidence at testing laboratory (mean ± SD): 68/745 (9% ± 5%); historical incidence in NTP studies: 205/2,519 (8% ± 5%)

Adrenal Gland: Pheochromocytomas in female mice occurred with significant positive trends, and the incidences in the dosed groups were significantly greater than those in the controls (Table 26). The pheochromocytomas varied in size and infiltrated into the cortex in a few animals. Cells were arranged as cords or as follicles and had granular, basophilic cytoplasm. Nuclei were vesicular or had coarse chromatin. Mitotic figures were not numerous.

Harderian Gland: Adenomas or cystadenomas (combined) in female mice occurred with a significant positive trend (Table 27). A carcinoma was observed in one control female mouse. However, the harderian glands in mice were only examined microscopically if they appeared abnormal at necropsy. In male mice, the number of adenomas or cystadenomas (combined) was 4/50 for control, 6/50 for low dose, and 4/50 for high dose mice.

Hematopoietic System: For both male and female mice, the incidences of all types of malignant lymphomas were not statistically significant by the Cochran-Armitage or Fisher exact tests (Table 28). However, lymphomas are considered lethal tumors, and in females there was a clear increase based on life table analyses.

TABLE 26. ANALYSIS OF ADRENAL GLAND TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	500 ppm	1,000 ppm
Pheochromocytoma			
Overall Rates	1/48 (2%)	7/47 (15%)	7/45 (16%)
Adjusted Rates	3.2%	32.4%	59.8%
Terminal Rates	1/31 (3%)	2/12 (17%)	3/6 (50%)
Life Table Tests	P<0.001	P=0.003	P<0.001
Cochran-Armitage Trend Test	P=0.025		
Fisher Exact Tests		P=0.027	P=0.024
Pheochromocytoma, Malignant			
Overall Rates	0/48 (0%)	1/47 (2%)	1/45 (2%)
Pheochromocytoma or Pheochromocytoma, Malignant (a)			
Overall Rates	1/48 (2%)	8/47 (17%)	8/45 (18%)
Adjusted Rates	3.2%	37.2%	73.2%
Terminal Rates	1/31 (3%)	2/12 (17%)	4/6 (67%)
Life Table Tests	P<0.001	P=0.001	P<0.001
Cochran-Armitage Trend Test	P=0.015		
Fisher Exact Tests		P=0.014	P=0.012

(a) Historical incidence at testing laboratory (mean ± SD): 5/704 (0.7% ± 1%); historical incidence in NTP studies: 15/2,357 (0.6% ± 1%)

TABLE 27. ANALYSIS OF HARDERIAN GLAND TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control (a)	500 ppm (a)	1,000 ppm (a)
Adenoma			
Overall Rates	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted Rates	0.0%	0.0%	27.6%
Terminal Rates	0/31 (0%)	0/12 (0%)	1/6 (17%)
Life Table Tests	P=0.004	(b)	P=0.011
Cochran-Armitage Trend Test	P=0.037		
Fisher Exact Tests		(b)	P=0.121
Cystadenoma			
Overall Rates	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adenoma or Cystadenoma			
Overall Rates	0/50 (0%)	0/50 (0%)	5/50 (10%)
Adjusted Rates	0.0%	0.0%	44.3%
Terminal Rates	0/31 (0%)	0/12 (0%)	2/6 (33%)
Life Table Tests	P<0.001	(b)	P<0.001
Cochran-Armitage Trend Test	P=0.006		
Fisher Exact Tests		(b)	P=0.028
Carcinoma			
Overall Rates	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adenoma, Cystadenoma, or Carcinoma (c)			
Overall Rates	1/50 (2%)	0/50 (0%)	5/50 (10%)
Adjusted Rates	2.5%	0.0%	44.3%
Terminal Rates	0/31 (0%)	0/12 (0%)	2/6 (33%)
Life Table Tests	P=0.002	P=0.628N	P=0.004
Cochran-Armitage Trend Test	P=0.037		
Fisher Exact Tests		P=0.500N	P=0.102

(a) The denominator is the number of animals on which a necropsy was performed.
(b) No P value is reported because no tumors were observed in the 500-ppm and control groups.
(c) Historical incidence at testing laboratory (mean ± SD): 14/748 (2% ± 1%); historical incidence in NTP studies: 33/2,537 (1% ± 2%)

TABLE 28. ANALYSIS OF HEMATOPOIETIC SYSTEM TUMORS IN MICE IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	500 ppm	1,000 ppm
MALE			
Lymphoma, All Malignant (a)			
Overall Rates	7/50 (14%)	9/50 (18%)	11/50 (22%)
Adjusted Rates	15.6%	23.7%	24.9%
Terminal Rates	5/42 (12%)	4/32 (13%)	5/36 (14%)
Life Table Tests	P=0.131	P=0.228	P=0.158
Incidental Tumor Tests	P=0.301	P=0.494	P=0.459
Cochran-Armitage Trend Test	P=0.181		
Fisher Exact Tests		P=0.393	P=0.218
FEMALE			
Lymphoma, All Malignant (b)			
Overall Rates	17/50 (34%)	24/50 (48%)	25/50 (50%)
Adjusted Rates	43.3%	74.1%	77.9%
Terminal Rates	10/31 (32%)	6/12 (50%)	2/6 (33%)
Life Table Tests	P<0.001	P<0.001	P=0.001
Cochran-Armitage Trend Test	P=0.065		
Fisher Exact Tests		P=0.111	P=0.078

(a) Historical incidence at testing laboratory (mean ± SD): 119/745 (16% ± 8%); historical incidence in NTP studies: 281/2,395 (12% ± 7%)
(b) Historical incidence at testing laboratory (mean ± SD): 232/748 (31% ± 13%); historical incidence in NTP studies: 637/2,537 (25% ± 10%)

IV. DISCUSSION AND CONCLUSIONS

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The toxicology and carcinogenicity of C.I. Basic Red 9 monohydrochloride was studied by giving the dye mixed with feed to F344/N rats and $B6C3F_1$ mice. The compound was first given at concentrations of 0, 250, 500, 1,000, 2,000, or 4,000 ppm to groups of 10 animals of each sex and species for 13 weeks. In addition to providing data for the selection of dietary concentrations for the 2-year studies, results from the 13-week studies indicated that the chemical produced effects in the thyroid and pituitary glands in rats. C.I. Basic Red 9 monohydrochloride exposure for 13 weeks caused enlarged thyroid glands, adenomatous goiter, and pituitary gland basophil hypertrophy in rats of each sex at the highest dose. However, any functional physiologic effects produced by the abnormal conditions of these two organs (which were seen grossly or microscopically) could not be determined.

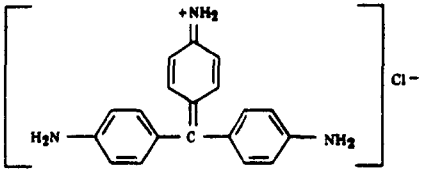
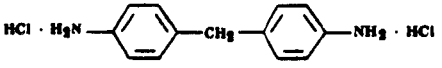
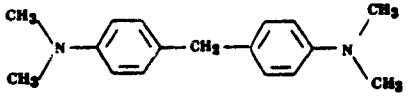
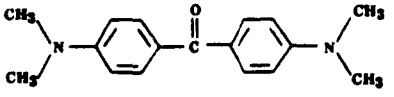
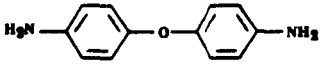
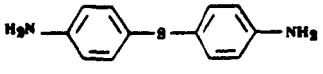
The pituitary and thyroid glands are intimately associated by a feedback mechanism that maintains appropriate levels of circulating thyroxine (T_4) and triiodothyronine (T_3), iodine-containing derivatives of the amino acid tyrosine. Thyroid-stimulating hormone (TSH) is secreted by the basophils of the anterior pituitary gland and promotes the uptake of iodine by the thyroid gland and the synthesis of the thyroid hormones. Under normal conditions, an excess of circulating T_3 and T_4 suppresses pituitary gland secretion of TSH and thyroid gland activity diminishes, whereas the anterior pituitary gland responds to low levels of circulating T_3 and T_4 by increasing the secretion of TSH and thereby promoting increased thyroid gland activity. One measure of a perturbation to the thyroid-pituitary feedback system would be a change in the plasma levels of T_4.

Long-term toxicology and carcinogenesis studies were conducted by administering C.I. Basic Red 9 monohydrochloride in feed to groups of 50 animals for 103 weeks at concentrations of 0, 1,000, or 2,000 ppm for male rats and 0, 500, or 1,000 ppm for female rats and mice of each sex. In these studies, 10 additional rats were included with the high dose and control groups to determine the effect of C.I. Basic Red 9 monohydrochloride on the thyroid and pituitary glands after 1 year and on circulating levels of T_4 at weeks 13, 26, 39, and 52.

Effects on the thyroid gland became apparent soon after exposure to C.I. Basic Red 9 monohydrochloride began, and they appeared to be similar in male and female rats. Male rats were exposed to the dye at dietary concentrations twice that for females, which may account for the greater response in males. For example, during exposure, serum T_4 levels of dosed males were significantly lower than those of the male controls at the first measurement after exposure began (13 weeks) and remained low throughout the study, whereas the serum T_4 levels of dosed females were significantly lower than those of the female controls only after week 26 and week 52; at 104 weeks, mean thyroid gland weights were significantly increased in dosed males but not in dosed females at week 52 when compared with the thyroid gland weights of controls. However, the final mean body weights for male and female rats (13% and 9%) were lower than those for the controls. No significant changes in the pituitary glands were observed in either sex.

After 1 year of exposure at 2,000 ppm, 1 male rat out of 10 had hyperplasia, adenoma, or carcinoma of the thyroid gland follicular epithelium, and one male (2,000 ppm) and two females (1,000 ppm) had thyroid gland follicular cysts. Exposure of rats to C.I. Basic Red 9 monohydrochloride for 2 years increased incidences of neoplastic lesions at several sites, including the thyroid gland follicular cells. Astwood et al. (1945) studied the changes in thyroid gland weight and iodine concentration in rats administered thioureylene and aminobenzene derivatives in the diet for 10 days compared with the effect of the antithyroid compound thiouracil. The investigators estimated the activity for basic fuschin to be 3% that of thiouracil. In the same study, 4,4'-diaminodiphenylmethane (4,4'-methylenedianiline) was one-fourth as active as thiouracil; 4,4'-methylenedianiline dihydrochloride was studied and found to cause thyroid gland neoplasms in F344/N rats and $B6C3F_1$ mice of each sex (NTP, 1983). Neoplastic lesions of the thyroid gland were also produced by the structurally related aromatic amines 4,4'-methylenebis(N,N-dimethyl)benzenamine (NCI, 1979a), 4,4'-oxydianiline (NCI, 1980), and 4,4'-thiodianiline (NCI, 1978) but not by Michler's ketone (NCI, 1979b) (Table 29). Thyroid gland hormones were not measured in any of these

TABLE 29. COMPARISON OF RESULTS OF TWO-YEAR NCI/NTP STUDIES ON C.I. BASIC RED 9 MONOHYDROCHLORIDE AND RELATED COMPOUNDS

Test Substance	Structure	Route of Exposure	Species (a)	Sex	Dose (ppm)	Site of Neoplastic Lesion: Liver	Site of Neoplastic Lesion: Thyroid Gland
C.I. Basic Red 9 monohydrochloride (current study)		Feed	Rat	M	2,000	+	+
				F	1,000		+
			Mouse	M	1,000	+	
				F	1,000	+	
4,4'-Methylenedianiline dihydrochloride (NTP, 1983)		Drinking water	Rat	M	300	+	+
				F	300		+
			Mouse	M	300	+	+
				F	300	+	+
4,4'-Methylenebis (N,N-dimethyl) benzenamine (NCI, 1979a)		Feed	Rat	M	750		+
				F	750		+
			Mouse	M	2,500		
				F	2,500	+	
Michler's Ketone (NCI, 1979b)		Feed	Rat	M	500	+	
				F	1,000	+	
			Mouse	M	2,500		
				F	2,500	+	
4,4'-Oxydianiline (NCI, 1980)		Feed	Rat	M	500	+	+
				F	500	+	+
			Mouse	M	800		
				F	800	+	+
4,4'-Thiodianiline (NCI, 1978)		Feed	Rat	M	3,000	+	+
				F	3,000		+
			Mouse	M	5,000	+	+
				F	5,000	+	+

(a) Rat: F344/N; mouse: $B6C3F_1$
(b) + = Neoplastic lesion occurred at statistically significant incidence ($P<0.025$ by the Fisher exact test).

studies. Of these related chemicals, only 4,4'-methylenebis(N,N-dimethyl)benzenamine did not produce lesions of the mouse thyroid glands. The relationships of these chemicals to thyroid gland function were discussed in the NTP Technical Report on 4,4'-methylenedianiline dihydrochloride (NTP, 1983).

Although organ weights were not recorded in the 2-year studies, the observations of enlarged thyroid glands in the high dose rats in the 13-week studies, follicular cell hyperplasia in high dose males in the 2-year studies (Table C1), and decreased T_4 in dosed animals in the 52-week supplemental study all support the hypothesis that secretion of TSH increases in response to the decreased circulating thyroid hormone levels. TSH stimulates the thyroid gland to increased thyroid hormone synthesis and discharge. However, if C.I. Basic Red 9 monohydrochloride interfered with thyroid hormone metabolism, TSH stimulation of the thyroid gland would continue, resulting in enlarged thyroid glands as a compensatory response to produce increased amounts of thyroid hormone.

None of the structurally related compounds produced skin lesions (see Table 29). In the present studies, neoplastic and nonneoplastic skin lesions occurred at increased incidences in high dose male but not in high dose female rats (see Table 12; Table C1). There is evidence of thyroid hormone involvement in the increase in keratin synthesis in vivo and in vitro in amphibian skin; this increase is apparently due to an increase in keratin mRNA (Reeves, 1977). Epidermal keratin mRNA has been identified and purified by Gibbs and Freedberg (1980, 1982) from the skin of guinea pigs and by Fuchs and Green (1980) from cultured human epidermal cells and rabbit epidermis. It is unclear if thyroid hormones are involved in de novo synthesis of keratin mRNA and subsequent keratin synthesis. In addition, thyroid hormones markedly affect many aspects of hair follicle activity, as is shown by experiments on sheep and rats as well as by clinical observations of humans (see Ebling and Hale, 1983).

Thyroid hormones, like the steroid hormones, are lipophilic, are bound to transport proteins in the plasma, and cross the cell membrane readily. Unlike steroid hormones, thyroid hormones are not carried by a cytosol receptor into the nucleus. Although nuclear receptors for thyroid hormones have been found in cells of several organs, these receptors have not been as well documented in the skin. However, at least one study has demonstrated the high affinity of T_3 for nuclear binding sites of fibroblasts grown from the skin of the deltoid region (Bernal et al., 1978). The aforementioned studies from the literature present evidence to suggest that skin and its appendages are affected by thyroid hormones and that there is probably some receptor(s) for these hormones at these sites. It is tempting to speculate that the C.I. Basic Red 9 monohydrochloride molecule or its metabolites are recognized both by the thyroid gland, such that there is competitive inhibition in the synthesis of T_3 or T_4, and by target-site thyroid hormone nuclear receptors.

Zymbal gland carcinomas in rats of each sex occurred with significant positive trends, and the incidences in the high dose groups were significantly greater ($P \leq 0.002$) than those in the controls. Necrosis and hyperkeratosis of this organ also appeared to be dose related in both sexes (Tables C1 and C2). Female rats, however, appeared to be more likely to develop Zymbal gland tumors at 1,000 ppm than were males at the same dose. Zymbal gland tumors have been shown to be associated with exposure to aromatic amines.

In the liver, focal and ischemic necrosis (Table C1) and the incidences of neoplastic nodules or carcinomas (combined) were increased in dosed male but not female rats. Neoplastic lesions in the liver also occurred in rats in the studies of the structurally related compounds previously discussed, except for 4,4'-methylenebis(N,N-dimethyl)benzenamine (see Table 29). In that study, the compound was administered for only 59 weeks to rats.

The results of exposure of $B6C3F_1$ mice to C.I. Basic Red 9 monohydrochloride at 0, 250, 500, 1,000, 2,000, or 4,000 ppm in the feed indicated no compound-related histopathologic effects after 13 weeks. Depressions in body weight gain were used as the basis for selection of doses of 0, 500, and 1,000 ppm for the 2-year studies. The 2-year studies of male mice had to be restarted after 9 months of exposure because excessive deaths were caused by fighting among cagemates. The male groups were restarted 11

months after the female groups were started, and the males in the restarted study were individually caged; only the results from the restarted study were complete and are presented in this report.

Survival of low dose male mice in the 2-year study was lower than that of the controls ($P = 0.032$), and the survival of low dose and high dose female mice was significantly lower than that of the controls ($P < 0.001$). Females also had a greater variety of significantly increased neoplastic lesions. The incidences of hepatocellular carcinomas in dosed male and female mice were significantly greater ($P < 0.001$) than those in the controls.

Chemically related incidences of neoplastic lesions at other sites occurred only in female mice. These lesions included adenomas or carcinomas (combined) of the lung in the high dose group. The mean historical incidences of alveolar/bronchiolar adenomas or carcinomas (combined) are 10% ± 5% at the testing laboratory and 7% ± 4% in NTP studies. Thus, the incidence of 11% in high dose female mice may not be a result of administration of the test chemical. (However, the survival of the high dose group was only 12% compared with a survival of historical controls of 60%.)

Female mice also had a greater incidence of adrenal gland pheochromocytomas than did controls. The incidence was well above historical values for both EG&G Mason Research Institute and other NTP studies (Appendix F, Table F19). Pheochromocytomas were recorded at incidences of 1/48 for control, 8/47 for low dose, and 8/45 for high dose female mice. Of these, 1 each of 23 low dose and 24 high dose mice that died before 90 weeks had this lesion.

C.I. Basic Red 9 monohydrochloride induced DNA damage (Rosenkranz and Poirier, 1979) and was mutagenic in bacteria (Dunkel, 1979; Appendix G, Table G1) and mammalian cells (Tables G2 and G3), induced sister-chromatid exchanges (Tables G4 and G5), and produced mutagenic urine in $B6C3F_1$ mice that had been exposed to the compound in feed at the concentrations used in the present study. In general, C.I. Basic Red 9 monohydrochloride required metabolic activation (rodent liver S9) in order to exhibit its genotoxic effects. Thus, one or more metabolites of C.I. Basic Red 9 monohydrochloride are likely responsible for the mutagenicity of this compound. A probable pathway that leads to activated C.I. Basic Red 9 monohydrochloride might involve N-hydroxylation of an amine followed by acetylation and/or conjugation. In the absence of more definitive information regarding the metabolism of C.I. Basic Red 9 monohydrochloride, it is not possible to ascribe the mutagenic activity of this compound to the formation of specific metabolites.

Conclusions: Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenicity** of C.I. Basic Red 9 monohydrochloride for male and female F344/N rats and for male and female $B6C3F_1$ mice. In male rats, C.I. Basic Red 9 monohydrochloride caused squamous cell carcinomas, trichoepitheliomas and sebaceous adenomas of the skin, subcutaneous fibromas, thyroid gland follicular cell adenomas and follicular cell carcinomas, Zymbal gland carcinomas, and hepatocellular carcinomas. In female rats, C.I. Basic Red 9 monohydrochloride caused subcutaneous fibromas, thyroid gland follicular cell adenomas or carcinomas (combined), and Zymbal gland carcinomas. In male mice, C.I. Basic Red 9 monohydrochloride caused hepatocellular carcinomas. In female mice, C.I. Basic Red 9 monohydrochloride caused hepatocellular carcinomas and adrenal gland pheochromocytomas or malignant pheochromocytomas (combined). Exposure to C.I. Basic Red 9 monohydrochloride also may have been related to increased incidences of mammary gland tumors in female rats and hematopoietic system tumors in female mice.

*Categories of evidence of carcinogenicity are defined in the Note to the Reader on page 2.

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V. REFERENCES

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS IN THE TWO-YEAR FEED STUDIES OF C. I. BASIC RED 9 MONOHYDROCHLORIDE

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT		2 (4%)	
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	2 (4%)	1 (2%)	4 (8%)
SQUAMOUS CELL CARCINOMA		1 (2%)	10 (20%)
BASAL-CELL CARCINOMA	1 (2%)		4 (8%)
TRICHOEPITHELIOMA			7 (14%)
SEBACEOUS ADENOMA			5 (10%)
KERATOACANTHOMA		1 (2%)	2 (4%)
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS	3 (6%)	2 (4%)	4 (8%)
FIBROMA	2 (4%)	20 (40%)	16 (32%)
FIBROSARCOMA	1 (2%)	3 (6%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
CARCINOMA, NOS, METASTATIC			1 (2%)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (6%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		1 (2%)
FOLLICULAR-CELL CARCINOMA, METAS		1 (2%)	
PHEOCHROMOCYTOMA, METASTATIC	1 (2%)		
FIBROSARCOMA, METASTATIC	1 (2%)		
OSTEOSARCOMA, METASTATIC	1 (2%)		1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
LEUKEMIA, MONONUCLEAR CELL	5 (10%)	1 (2%)	1 (2%)
*HEMATOPOIETIC SYSTEM	(50)	(50)	(50)
LEUKEMIA, NOS	2 (4%)		
CIRCULATORY SYSTEM			
#SPLEEN	(49)	(49)	(49)
HEMANGIOMA			1 (2%)
DIGESTIVE SYSTEM			
*MOUTH	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA			1 (2%)
*ORAL MUCOUS MEMBRANE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
#LIVER	(50)	(50)	(50)
BILE DUCT ADENOMA			1 (2%)
BILE DUCT CARCINOMA			2 (4%)
NEOPLASTIC NODULE	5 (10%)	14 (28%)	6 (12%)
HEPATOCELLULAR CARCINOMA		2 (4%)	8 (16%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM (Continued)			
#PANCREAS	(47)	(50)	(46)
ACINAR-CELL ADENOMA	1 (2%)	2 (4%)	1 (2%)
#STOMACH	(48)	(50)	(48)
ADENOCARCINOMA, NOS	1 (2%)		
#DUODENUM	(44)	(48)	(42)
ADENOCARCINOMA, NOS			1 (2%)
#JEJUNUM AND ILEUM COMBINED SITE	(44)	(48)	(42)
ADENOCARCINOMA, NOS	1 (2%)		
#COLON	(45)	(45)	(42)
ADENOCARCINOMA, NOS			2 (5%)
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(49)
TUBULAR-CELL ADENOMA		1 (2%)	
LIPOSARCOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(47)	(46)
CARCINOMA, NOS		1 (2%)	
ADENOMA, NOS	17 (35%)	16 (34%)	8 (17%)
#ADRENAL	(49)	(49)	(48)
CORTICAL ADENOMA		1 (2%)	
PHEOCHROMOCYTOMA	10 (20%)	14 (29%)	3 (6%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)		
#THYROID	(49)	(46)	(44)
FOLLICULAR-CELL ADENOMA			9 (20%)
FOLLICULAR-CELL CARCINOMA		5 (11%)	18 (41%)
C-CELL ADENOMA	4 (8%)	2 (4%)	
C-CELL CARCINOMA		1 (2%)	1 (2%)
#PANCREATIC ISLETS	(47)	(50)	(46)
ISLET-CELL ADENOMA	2 (4%)	2 (4%)	3 (7%)
ISLET-CELL CARCINOMA		3 (6%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOMA, NOS	1 (2%)		
FIBROADENOMA	4 (8%)	6 (12%)	2 (4%)
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA, NOS	1 (2%)		1 (2%)
ADENOMA, NOS	3 (6%)	2 (4%)	1 (2%)
#TESTIS	(48)	(48)	(50)
INTERSTITIAL-CELL TUMOR	43 (90%)	46 (96%)	37 (74%)
NERVOUS SYSTEM			
#BRAIN	(49)	(50)	(50)
CARCINOMA, NOS, INVASIVE		1 (2%)	
ASTROCYTOMA	1 (2%)		1 (2%)
SPECIAL SENSE ORGANS			
*EXTERNAL EAR	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA			1 (2%)
*ZYMBAL GLAND	(50)	(50)	(50)
CARCINOMA, NOS	1 (2%)	1 (2%)	13 (26%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(50)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
*THORACIC CAVITY	(50)	(50)	(50)
MESOTHELIOMA, INVASIVE		1 (2%)	
*ABDOMINAL CAVITY	(50)	(50)	(50)
SARCOMA, NOS			1 (2%)
OSTEOSARCOMA			1 (2%)
*TUNICA VAGINALIS	(50)	(50)	(50)
MESOTHELIOMA, NOS	1 (2%)	2 (4%)	1 (2%)
MESOTHELIOMA, MALIGNANT		1 (2%)	
ALL OTHER SYSTEMS			
MULTIPLE SITES			
MESOTHELIOMA, NOS		1	
*MULTIPLE ORGANS	(50)	(50)	(50)
HEPATOCELLULAR CARCINOMA, INVASI			1 (2%)
SARCOMA, NOS, INVASIVE		1 (2%)	
SARCOMA, NOS, METASTATIC		1 (2%)	1 (2%)
SARCOMA, NOS, UNC PRIM OR META			1 (2%)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)
MESOTHELIOMA, METASTATIC		1 (2%)	
CHEEK			
SQUAMOUS CELL PAPILLOMA			1
ORBITAL REGION			
FIBROSARCOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	10	13	29
MORIBUND SACRIFICE	7	9	21
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	33	28	
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			

@ INCLUDES AUTOLYZED ANIMALS

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	48	50	46
TOTAL PRIMARY TUMORS	116	160	186
TOTAL ANIMALS WITH BENIGN TUMORS	48	49	42
TOTAL BENIGN TUMORS	89	117	106
TOTAL ANIMALS WITH MALIGNANT TUMORS	17	21	34
TOTAL MALIGNANT TUMORS	21	25	70
TOTAL ANIMALS WITH SECONDARY TUMORS##	3	5	6
TOTAL SECONDARY TUMORS	3	7	7
TOTAL ANIMALS WITH TUMORS UNCERTAIN-- BENIGN OR MALIGNANT	6	15	9
TOTAL UNCERTAIN TUMORS	6	18	9
TOTAL ANIMALS WITH TUMORS UNCERTAIN-- PRIMARY OR METASTATIC			1
TOTAL UNCERTAIN TUMORS			1

* NUMBER OF ANIMALS NECROPSIED
** PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
BASAL-CELL CARCINOMA	1 (2%)		1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS	1 (2%)	2 (4%)	2 (4%)
FIBROMA		15 (30%)	10 (20%)
FIBROSARCOMA		2 (4%)	
CARCINOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	
PHEOCHROMOCYTOMA, METASTATIC		1 (2%)	
SARCOMA, NOS, METASTATIC			1 (2%)
LIPOSARCOMA, METASTATIC	1 (2%)		
CARCINOSARCOMA, METASTATIC		1 (2%)	
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (2%)	
MALIG. LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
LEUKEMIA, MONONUCLEAR CELL	10 (20%)	11 (22%)	6 (12%)
*HEMATOPOIETIC SYSTEM	(50)	(50)	(50)
LEUKEMIA, NOS	1 (2%)		
#SPLEEN	(49)	(49)	(50)
LIPOMA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
NEOPLASTIC NODULE	1 (2%)	4 (8%)	3 (6%)
HEPATOCELLULAR CARCINOMA			1 (2%)
PHEOCHROMOCYTOMA, METASTATIC		1 (2%)	
ENDOMETRIAL STROMAL SARCOMA, MET			1 (2%)
#ILEUM	(49)	(50)	(49)
ADENOCARCINOMA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
TUBULAR-CELL ADENOMA		1 (2%)	
#URINARY BLADDER	(48)	(50)	(49)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	

TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(50)	(48)	(49)
CARCINOMA, NOS	1 (2%)	2 (4%)	1 (2%)
ADENOMA, NOS	26 (52%)	20 (42%)	21 (43%)
#ADRENAL	(50)	(50)	(49)
CORTICAL ADENOMA		1 (2%)	2 (4%)
CORTICAL CARCINOMA		1 (2%)	
PHEOCHROMOCYTOMA		2 (4%)	1 (2%)
PHEOCHROMOCYTOMA, MALIGNANT		1 (2%)	
#THYROID	(47)	(48)	(50)
FOLLICULAR-CELL ADENOMA			4 (8%)
FOLLICULAR-CELL CARCINOMA		2 (4%)	2 (4%)
C-CELL ADENOMA	1 (2%)	3 (6%)	1 (2%)
C-CELL CARCINOMA	1 (2%)	2 (4%)	1 (2%)
#PANCREATIC ISLETS	(49)	(47)	(49)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOMA, NOS		3 (6%)	1 (2%)
ADENOCARCINOMA, NOS	2 (4%)	2 (4%)	5 (10%)
FIBROADENOMA	22 (44%)	31 (62%)	29 (58%)
*CLITORAL GLAND	(50)	(50)	(50)
CARCINOMA, NOS	1 (2%)	1 (2%)	2 (4%)
SQUAMOUS CELL CARCINOMA	1 (2%)		
ADENOMA, NOS	2 (4%)		3 (6%)
#UTERUS	(50)	(50)	(49)
ADENOCARCINOMA, NOS			2 (4%)
ENDOMETRIAL STROMAL POLYP	15 (30%)	14 (28%)	10 (20%)
ENDOMETRIAL STROMAL SARCOMA	1 (2%)	5 (10%)	6 (12%)
CARCINOSARCOMA		1 (2%)	
FIBROADENOMA			1 (2%)
#CERVIX UTERI	(50)	(50)	(49)
SQUAMOUS CELL CARCINOMA	1 (2%)		
#UTERUS/ENDOMETRIUM	(50)	(50)	(49)
CARCINOMA, NOS		2 (4%)	1 (2%)
#OVARY	(49)	(49)	(47)
GRANULOSA-CELL TUMOR		2 (4%)	
NERVOUS SYSTEM			
#BRAIN	(50)	(49)	(50)
ASTROCYTOMA	2 (4%)		1 (2%)
SPECIAL SENSE ORGANS			
*ZYMBAL GLAND	(50)	(50)	(50)
CARCINOMA, NOS		2 (4%)	7 (14%)
MUSCULOSKELETAL SYSTEM			
*VERTEBRA	(50)	(50)	(50)
LIPOSARCOMA	1 (2%)		
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
ENDOMETRIAL STROMAL SARCOMA, INV			2 (4%)
*MESENTERY	(50)	(50)	(50)
FIBROMA		1 (2%)	

TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
SARCOMA, NOS, UNC PRIM OR META			1 (2%)
CARCINOSARCOMA, INVASIVE		1 (2%)	
DIAPHRAGM			
CARCINOSARCOMA, METASTATIC		1	
LEG			
OSTEOSARCOMA		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	5	13	20
MORIBUND SACRIFICE	8	6	18
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	37	31	12
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	41	50	48
TOTAL PRIMARY TUMORS	93	139	130
TOTAL ANIMALS WITH BENIGN TUMORS	37	43	43
TOTAL BENIGN TUMORS	67	93	84
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	30	29
TOTAL MALIGNANT TUMORS	25	40	42
TOTAL ANIMALS WITH SECONDARY TUMORS##	1	3	4
TOTAL SECONDARY TUMORS	1	6	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-- BENIGN OR MALIGNANT	1	6	3
TOTAL UNCERTAIN TUMORS	1	6	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-- PRIMARY OR METASTATIC			1
TOTAL UNCERTAIN TUMORS			1

* NUMBER OF ANIMALS NECROPSIED
** PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF C. I. BASIC RED 9 MONOHYDROCHLORIDE: UNTREATED CONTROL

ANIMAL NUMBER	0011	0012	0013	0014	0015	0016	0017	0018	0019	0020	0021	0022	0023	0024	0025	0026	0027	0028	0029	0030	0031	0032	0033	0034	0035
WEEKS ON STUDY	104	092	075	091	108	108	106	108	108	108	106	106	108	108	108	106	108	108	092	106	105	083	108	108	062
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																X									
Basal cell carcinoma										X															
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS			X											X											
Fibroma									X																
Fibrosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									
Pheochromocytoma, metastatic																									
Fibrosarcoma, metastatic																							X		
Osteosarcoma, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	–	+	–	+	+	–	+	+	+	+	+	+	–	+	+	+	+	–	+	–	–	+	–	–	
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma																									
Salivary gland	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule									X			X											X		
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Adenocarcinoma, NOS																									
Small intestine	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+
Adenocarcinoma, NOS																									
Large intestine	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS		X				X		X	X							X			X	X	X	X			
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma								X			X	X	X			X									
Pheochromocytoma, malignant																									
Thyroid	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma								X	X																
Parathyroid	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+
Pancreatic islets	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma															X			X							
REPRODUCTIVE SYSTEM																									
Mammary gland	+	N	N	N	N	N	+	N	N	N	N	N	+	N	+	N	N	N	N	N	N	+	N	N	
Adenoma, NOS																									
Fibroadenoma	X																					X			
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+		
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	–	X	X		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+		
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
Carcinoma, NOS										N															
Adenoma, NOS						X				X															
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+		
Astrocytoma	X																								

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	022	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	108	106	106	103	106	106	106	106	106	106	080	106	105	106	092	106	106	081	106	101	095	106	101	106	106	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma	X																									2
Basal cell carcinoma																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS											X															3
Fibroma																					X					2
Fibrosarcoma																					X					1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma															X											1
Pheochromocytoma, metastatic												X														1
Fibrosarcoma, metastatic																										1
Osteosarcoma, metastatic											X															1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	49
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	–	+	+	–	+	+	+	–	+	+	+	–	+	+	–	+	–	–	–	–	–	+	31
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell carcinoma								X																		1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule									X					X												3
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	47
Acinar cell adenoma					X																					1
Esophagus	+	+	+	+	+	+	+	–	–	+	+	+	+	+	–	–	+	+	+	+	+	+	+	–	+	45
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	48
Adenocarcinoma, NOS																									X	1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	44
Adenocarcinoma, NOS					X																					1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	46
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	47
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–	+	+	+	+	+	48
Adenoma, NOS			X						X			X			X		X					X		X	X	17
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	49
Pheochromocytoma	X				X						X		X			X										10
Pheochromocytoma, malignant												X														1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell adenoma			X																X							4
Parathyroid	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	+	+	+	–	+	+	13
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	47
Islet cell adenoma																										2
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	+	N	N	+	+	+	*50
Adenoma, NOS																									X	1
Fibroadenoma				X																			X			4
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	48
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X		X	X	X		X	X	X	X		X		X	X	X	43
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	48
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
Adenoma, NOS					X	X																				3
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Astrocytoma																										1

* Animals Necropsied

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF C. I. BASIC RED 9 MONOHYDROCHLORIDE: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	104	092	075	091	106	106	106	106	106	106	106	106	106	106	106	106	106	106	098	106	105	083	106	106	062
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
BODY CAVITIES																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+
Mesothelioma, NOS														X											
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell				X																	X			X	
Orbital region																									
Fibrosarcoma																								X	
Hematopoietic system																									
Leukemia, NOS																									

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	106	106	106	093	106	106	106	106	106	106	060	106	105	106	098	106	106	081	106	111	095	106	101	106	106	
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS															X											1
BODY CAVITIES																										
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	*50
Mesothelioma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell					X								X													5
Orbital region																										
Fibrosarcoma																										1
Hematopoietic system																										
Leukemia, NOS																		X			X					2

* Animals Necropsied

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: LOW DOSE

ANIMAL NUMBER	0001	0002	0003	0004	0005	0006	0007	0008	0009	0010	0011	0012	0013	0014	0015	0016	0017	0018	0019	0020	0021	0022	0023	0024	0025
WEEKS ON STUDY	087	105	105	088	082	105	089	092	105	105	105	105	105	105	101	103	105	105	105	105	105	104	105	090	105
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	N	+	+	N	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Squamous cell carcinoma																									
Keratoacanthoma												X													
Subcutaneous tissue	+	+	+	N	+	+	N	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																								X	
Fibroma	X	X			X			X			X		X			X		X			X				X
Fibrosarcoma													X										X		
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic																									
Alveolar/bronchiolar adenoma				X																					
Follicular cell carcinoma, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	–	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule		X	X								X			X		X					X		X	
Hepatocellular carcinoma														X											
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	–	–	+	+	–	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																									X
Liposarcoma																									
Urinary bladder	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE																									
Pituitary	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Carcinoma, NOS																									
Adenoma, NOS	X	X	X		X		X						X	X	X			X							
Adrenal	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																									X
Pheochromocytoma			X			X			X	X		X								X					
Thyroid	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma														X	X										
C-cell adenoma																									
C-cell carcinoma			X																						
Parathyroid	–	–	–	–	+	+	–	–	–	+	–	+	+	–	–	–	–	–	+	–	–	–	+	+	–
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma							X																		
Islet cell carcinoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	+	N	N	+	N	N	+	+	N	N	N	N	N	N	+	N	+	N	N	N	N	+	+
Fibroadenoma						X			X										X						
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS	X																								
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, invasive																									

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE
(Continued)

ANIMAL NUMBER	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	101	105	091	103	105	088	105	094	105	105	095	095	105	104	063	105	105	105	105	105	093	105	105	054	095	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma																						X				1
Squamous cell carcinoma					X																					1
Keratoacanthoma																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS	X																									2
Fibroma			X			X				X	X	X			X	X							X		X	20
Fibrosarcoma		X																								3
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic					X																					1
Alveolar/bronchiolar adenoma					X					X																3
Follicular cell carcinoma, metastatic																					X					1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	49
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	49
Thymus	–	+	–	–	–	+	+	+	+	+	–	+	+	–	–	+	+	–	–	+	–	+	+	–	+	34
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule		X				X				X								X				X	X		X	14
Hepatocellular carcinoma					X																					2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinar cell adenoma		X	X																							2
Esophagus	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	41
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	48
Large intestine	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma																										1
Liposarcoma											X															1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	47
Carcinoma, NOS				X																						1
Adenoma, NOS	X					X	X	X	X	X			X													16
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cortical adenoma																										1
Pheochromocytoma		X								X	X	X	X							X		X	X			14
Thyroid	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	46
Follicular cell carcinoma													X								X		X			5
C-cell adenoma	X				X																					2
C-cell carcinoma																										1
Parathyroid	+	–	–	+	+	–	–	+	+	+	–	–	+	–	–	–	–	–	+	–	–	+	+			19
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Islet cell adenoma													X													2
Islet cell carcinoma		X				X										X										3
REPRODUCTIVE SYSTEM																										
Mammary gland	+	N	N	N	N	N	N	N	+	+	N	N	N	N	N	+	N	N	N	N	+	N	+	N	N	*50
Fibroadenoma																X					X		X			6
Testis	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Interstitial cell tumor		X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	46
Prostate	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	48
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS			X																							2
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, invasive				X																						1

* Animals Necropsied

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: LOW DOSE (Continued)

ANIMAL NUMBER	091	092	093	094	095	096	097	098	099	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125
WEEKS ON STUDY	087	105	105	085	082	105	089	092	105	105	105	105	105	105	101	105	105	105	105	105	105	104	105	090	105
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS									X																
BODY CAVITIES																									
Pleura	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	X	N	N	N
Mesothelioma, invasive																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, NOS		X																							
Mesothelioma, malignant																						X			
ALL OTHER SYSTEMS																									
Multiple sites, NOS																					X				
Mesothelioma, NOS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS, invasive																								X	
Sarcoma, NOS, metastatic																								X	
Fibrous histiocytoma, malignant				X																					
Mesothelioma, NOS	X																								
Mesothelioma, metastatic																						X			
Malignant lymphoma, NOS																									
Leukemia, mononuclear cell																									

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	022	027	028	029	032	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL:
WEEKS ON STUDY	101	109	091	103	108	088	105	092	095	095	095	095	092	094	083	095	095	095	095	095	093	095	095	054	095	TISSUES TUMORS
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
BODY CAVITIES																										
Pleura	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
Mesothelioma, invasive																										1
Tunica vaginalis	N	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Mesothelioma, NOS																							X			2
Mesothelioma, malignant																										1
ALL OTHER SYSTEMS																										
Multiple sites, NOS																										
Mesothelioma, NOS																										1
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS, invasive																										1
Sarcoma, NOS, metastatic																										1
Fibrous histiocytoma, malignant																						X				2
Mesothelioma, NOS																										1
Mesothelioma, metastatic																										1
Malignant lymphoma, NOS			X																							1
Leukemia, mononuclear cell																		X								1

* Animals Necropsied

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: HIGH DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	058	116	162	187	118	190	166	191	188	194	166	185	104	117	174	171	166	184	173	162	180	199	195	104	143
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																		X							
Squamous cell carcinoma			X						X												X		X	X	
Basal cell carcinoma								X												X	X				
Trichoepithelioma								X		X										X			X		
Sebaceous adenoma									X																
Keratoacanthoma																						X			
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS													X						X						
Fibroma			X				X	X					X									X	X	X	
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic															X										
Squamous cell carcinoma, metastatic																									
Hepatocellular carcinoma, metastatic								X																	
Alveolar/bronchiolar adenoma											X					X									
Alveolar/bronchiolar carcinoma																					X				
Osteosarcoma, metastatic																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Hemangioma										X															
Lymph nodes	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	–	+	+	–	–	–	–	–	+	–	–	–	+	–	–	+	–	+	+	+	+	+	+	+	–
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	X	N	N	N
Squamous cell papilloma																									
Salivary gland	+	+	+	+	+	+	+	+	–	+	+	–	+	+	–	+	+	–	+	–	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct adenoma																									
Bile duct carcinoma																									
Neoplastic nodule			X													X					X	X			
Hepatocellular carcinoma																							X		
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	–
Acinar cell adenoma							X																		
Esophagus	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–
Small intestine	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	–
Adenocarcinoma, NOS																									
Large intestine	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	–
Adenocarcinoma, NOS												X													
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Urinary bladder	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+
Adenoma, NOS																				X					
Adrenal	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma				X													X								
Thyroid	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–
Follicular cell adenoma							X		X	X								X			X		X		
Follicular cell carcinoma			X	X		X					X				X	X	X					X		X	
C-cell carcinoma																									
Parathyroid	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	–
Islet cell adenoma																							X		
Islet cell carcinoma									X																
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	N	N	N	N	N	+	N	N	N	+	N	N	N	N	N	N	N	N	N	+	N	+
Fibroadenoma													X												
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor				X		X	X	X	X	X	X	X	X		X			X			X	X	X	X		
Prostate	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
Adenoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma																X									

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: : No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	088	098	086	100	071	097	063	073	075	081	072	074	078	078	082	085	097	072	060	075	093	083	077	087	094	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	*50
Squamous cell papilloma		X								X					X											4
Squamous cell carcinoma			X	X		X															X	X				10
Basal cell carcinoma																	X									4
Trichoepithelioma																									X	7
Sebaceous adenoma																									X	5
Keratoacanthoma			X																							2
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	
Sarcoma, NOS																X						X				4
Fibroma	X					X				X				X	X		X			X	X			X		16
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																										1
Squamous cell carcinoma, metastatic				X																						1
Hepatocellular carcinoma, metastatic																X										2
Alveolar/bronchiolar adenoma																							X			3
Alveolar/bronchiolar carcinoma																										1
Osteosarcoma, metastatic																							X			1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangioma																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	48
Thymus	-	-	+	-	-	-	-	-	+	+	+	-	-	+	-	-	+	-	-	-	+	+	-	+	+	22
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell papilloma																										1
Salivary gland	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	40
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct adenoma															X											1
Bile duct carcinoma						X															X					2
Neoplastic nodule										X										X						6
Hepatocellular carcinoma						X									X	X	X				X					8
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	*50
Pancreas	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Acinar cell adenoma																										1
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	44
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	-	+	+	42
Adenocarcinoma, NOS																					X					1
Large intestine	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	42
Adenocarcinoma, NOS														X												2
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoma, NOS		X				X		X									X	X			X	X				8
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	48
Pheochromocytoma																					X					3
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	44
Follicular cell adenoma						X															X		X			9
Follicular cell carcinoma		X						X	X				X	X	X						X	X			X	18
C-cell carcinoma																	X									1
Parathyroid	+	-	+	-	-	-	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	10
Pancreatic islets	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Islet cell adenoma						X																			X	3
Islet cell carcinoma																										1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	N	N	+	N	+	N	N	+	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	*50
Fibroadenoma	X																									2
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	37
Prostate	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS			X																							1
Adenoma, NOS										X																1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma																										1

*Animals Necropsied

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF C. I. BASIC RED 9, MONOHYDROCHLORIDE: HIGH DOSE (Continued)

ANIMAL NUMBER	021	022	023	024	025	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045
WEEKS ON STUDY	052	076	082	087	089	092	096	098	102	102	104	106	106	106	106	106	106	106	106	106	106	106	106	104	103
SPECIAL SENSE ORGANS																									
Ear	+	N	N	N	N	+	N	+	N	+	N	N	N	N	+	N	+	N	N	N	N	N	+	+	N
Squamous cell papilloma																									
Zymbal gland	+	N	N	N	N	+	N	+	N	+	N	N	N	N	+	N	+	N	N	N	N	N	+	+	N
Carcinoma, NOS	X					X		X		X					X		X						X	X	
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS																									
Osteosarcoma																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, NOS																									
Body cavities	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hepatocellular carcinoma, invasive																									
Sarcoma, NOS, metastatic																									
Sarcoma, NOS, uncprim or metastatic												X													
Mesothelioma, NOS																									
Leukemia, mononuclear cell																									
Cheek, NOS																									
Squamous cell papilloma																									

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	0226	0227	0228	0229	0230	0231	0232	0233	0234	0235	0236	0237	0238	0239	0240	0241	0242	0243	0244	0245	0246	0247	0248	0249	0250	TOTAL:
WEEKS ON STUDY	0288	0293	0296	1010	0711	0297	0633	0713	0715	0851	0712	0714	0718	0718	0822	0825	0917	0722	0600	0715	0923	0933	0717	0837	0894	TISSUES TUMORS
SPECIAL SENSE ORGANS																										
Ear	+	+	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	+	+	N	N	N	+	*50	
Squamous cell papilloma																								X	1	
Zymbal gland	+	+	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	+	+	N	N	N	+	*50	
Carcinoma, NOS	X	X											X						X	X					13	
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50	
Sarcoma, NOS				X																					1	
Osteosarcoma																							X		1	
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50	
Mesothelioma, NOS	X																								1	
Body cavities	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50	
Mesothelioma, NOS					X																				1	
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50	
Hepatocellular carcinoma, invasive																X									1	
Sarcoma, NOS, metastatic																								X	1	
Sarcoma, NOS, uncertain prim or metastatic																									1	
Mesothelioma, NOS							X																		1	
Leukemia, mononuclear cell			X																						1	
Cheek, NOS																										
Squamous cell papilloma					X																				1	

*Animals Necropsied

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: UNTREATED CONTROL

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	096	101	106	106	106	106	085	106	106	106	106	106	106	086	106	106	106	106	106	106	106	106	106	106	106
INTEGUMENTARY SYSTEM																									
Skin	+	N	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Subcutaneous tissue	+	N	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Sarcoma, NOS																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Liposarcoma, metastatic														X											
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Thymus	–	+	+	+	+	+	–	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Neoplastic nodule																						X			
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Large intestine	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Carcinoma, NOS								X																	
Adenoma, NOS	X	X		X	X	X				X	X	X			X		X			X	X	X	X	X	X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
C-cell adenoma															X										
C-cell carcinoma																									
Parathyroid	+	–	–	–	–	–	+	–	–	–	–	–	+	+	–	+	–	+	–	–	–	–	–	–	
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	N	+	N	+	+	+	+	+	+	N	+	N	+	N	+	+	N	+	N	+	+	N	N
Adenocarcinoma, NOS				X							X														
Fibroadenoma		X					X	X	X	X	X		X		X		X	X		X		X	X		
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																		X							
Squamous cell carcinoma						X																			
Adenoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Squamous cell carcinoma																									
Endometrial stromal polyp						X		X		X					X	X		X					X		
Endometrial stromal sarcoma																									
Ovary	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Astrocytoma																		X							
MUSCULOSKELETAL SYSTEM																									
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Liposarcoma														X											
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS																									
Leukemia, mononuclear cell	X						X						X								X	X			
Hematopoietic system																									
Leukemia, NOS		X																							

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL:
WEEKS ON STUDY	106	086	096	106	096	079	106	093	096	087	096	096	092	091	096	096	096	101	096	096	096	096	079	096	106	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Basal cell carcinoma																	X									1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS			X																							1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liposarcoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	–	+	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	42
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Pancreas	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS																										1
Adenoma, NOS	X			X				X		X			X			X	X	X	X	X						26
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid	+	+	+	+	+	–	+	+	+	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	47
C-cell adenoma																										1
C-cell carcinoma																									X	1
Parathyroid	+	–	–	–	–	–	+	+	–	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	14
Pancreatic islets	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islet cell adenoma																							X			1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	N	N	+	N	+	+	N	N	+	N	+	+	N	N	N	+	+	+	N	N	+	+	N	+	*50
Adenocarcinoma, NOS																										2
Fibroadenoma	X						X			X			X				X	X	X				X		X	22
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
Squamous cell carcinoma																										1
Adenoma, NOS							X										X									2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																			X							1
Endometrial stromal polyp			X						X		X						X	X			X	X		X		15
Endometrial stromal sarcoma																			X							1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma													X													2
MUSCULOSKELETAL SYSTEM																										
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Liposarcoma																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, NOS																			X							1
Leukemia, mononuclear cell		X						X					X					X						X		10
Hematopoietic system																										
Leukemia, NOS																										1

* *Animals Necropsied*

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: LOW DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	105	105	092	105	105	105	105	105	104	105	105	101	094	105	105	082	091	106	106	105	106	106	100	106	105
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS			·																				X		
Fibroma		X	·		X			X				X	X	X				X	X		X				
Fibrosarcoma												X													
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma		X														X									
Pheochromocytoma, metastatic																									X
Carcinosarcoma, metastatic																									
Osteosarcoma, metastatic																	X								
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																				X					
Pheochromocytoma, metastatic				·																					X
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma								X																	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional cell carcinoma																									
ENDOCRINE SYSTEM																									
Pituitary	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS				X			X			X	X	X	X		X			X		X	X	X	X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																									
Cortical carcinoma																									
Pheochromocytoma									·																
Pheochromocytoma, malignant																									X
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																									
C-cell adenoma								X																	
C-cell carcinoma																					X				
Parathyroid	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	N	+	+	+	N	N	+	+	+	N	+	+	N	+	N
Adenoma, NOS		X	X																						
Adenocarcinoma, NOS		X								X															
Fibroadenoma		X	X	X	X	X		X	X			X	X	X				X	X		X	X		X	
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																								X	
Endometrial stromal polyp		X	X				X				X	X		X					X				X	X	
Endometrial stromal sarcoma	X						X															X			
Carcinosarcoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor											X														
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+

+ : Tissue Examined Microscopically
- : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: : No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	094	103	106	106	106	101	106	103	106	106	106	106	097	106	106	103	100	096	094	106	106	106	106	106	106	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																	X									2
Fibroma								X			X				X		X							X	X	15
Fibrosarcoma		X																								2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										2
Pheochromocytoma, metastatic																										1
Carcinosarcoma, metastatic																						X				1
Osteosarcoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	49
Lymph nodes	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	–	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	42
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule			X					X			X															4
Pheochromocytoma, metastatic																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	+	+	+	47
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional cell carcinoma	X																									1
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, NOS																					X		X			2
Adenoma, NOS		X	X				X								X	X	X		X	X						20
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cortical adenoma			X																							1
Cortical carcinoma										X																1
Pheochromocytoma				X		X																				2
Pheochromocytoma, malignant																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	48
Follicular cell carcinoma																	X						X			2
C-cell adenoma													X	X												3
C-cell carcinoma										X																2
Parathyroid	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+	–	+	–	+	+	–	–	–	+	13
REPRODUCTIVE SYSTEM																										
Mammary gland	+	N	+	+	+	+	+	N	N	+	+	+	N	+	+	+	+	N	+	+	+	+	+	+	+	*50
Adenoma, NOS											X															3
Adenocarcinoma, NOS																										2
Fibroadenoma	X		X	X	X	X	X				X	X			X	X			X	X	X		X	X	X	31
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS						X																				1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS				X																						2
Endometrial stromal polyp				X				X				X										X	X			14
Endometrial stromal sarcoma	X													X												5
Carcinosarcoma																						X				1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	49
Granulosa cell tumor																							X			2
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

*Animals Necropsied

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: LOW DOSE (Continued)

ANIMAL NUMBER	0901	0902	0903	0904	0905	0906	0907	0908	0909	0910	0911	0912	0913	0914	0915	0916	0917	0918	0919	0920	0921	0922	0923	0924	0925
WEEKS ON STUDY	105	105	098	105	103	105	105	105	104	105	101	094	105	105	082	091	108	108	105	106	108	110	106	106	105
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	+	N	N	N	N	N	N	N	N	N
Carcinoma, NOS													X			X									
BODY CAVITIES																									
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibroma					X																				
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrous histiocytoma, malignant																									
Carcinosarcoma, invasive																									
Malignant lymphoma, NOS																									
Leukemia, mononuclear cell		X				X			X																
Diaphragm, NOS																									
Carcinosarcoma, metastatic																									
Leg, NOS																									
Osteosarcoma																	X								

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	0226	0227	0228	0229	0230	0231	0232	0233	0234	0235	0236	0237	0238	0239	0240	0241	0242	0243	0244	0245	0246	0247	0248	0249	0250	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	102	106	106	101	106	103	106	106	106	106	107	102	106	106	103	100	102	104	106	106	102	106	106	106	
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										2
BODY CAVITIES																										
Mesentery	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Fibroma																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Fibrous histiocytoma, malignant									X																	1
Carcinosarcoma, invasive																					X					1
Malignant lymphoma, NOS																				X						1
Leukemia, mononuclear cell								X		X	X	X					X	X	X				X			11
Diaphragm, NOS																										
Carcinosarcoma, metastatic																					X					1
Leg, NOS																										
Osteosarcoma																										1

* Animals Necropsied

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: HIGH DOSE

ANIMAL NUMBER	0012	0023	0034	0045	0056	0067	0078	0089	0090	0111	0122	0133	0144	0155	0168	0177	0189	0200	0211	0222	0233	0244	0255
WEEKS ON STUDY	0833	1004	1104	1104	1004	0963	0833	1004	0833	1202	0871	1005	1101	1033	0922	1005	1104	1005	0870	0900	0871	0996	1005
INTEGUMENTARY SYSTEM																							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																							
Basal cell carcinoma																							
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS														X									
Fibroma				X		X		X			X		X							X			
Carcinosarcoma																							
RESPIRATORY SYSTEM																							
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS, metastatic																							
Sarcoma, NOS, metastatic														X									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																							
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma														X									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	A	+	+	+	+	+	–	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	–
CIRCULATORY SYSTEM																							
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																							
Salivary gland	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule															X								
Hepatocellular carcinoma																							
Endometrial stromal sarcoma, metasta															X								
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS								X															
Large intestine	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																							
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																							
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																	X						
Adenoma, NOS			X			X				X	X		X					X	X		X		X
Adrenal	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma														X									X
Pheochromocytoma																							
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma											X												
Follicular cell carcinoma																		X					
C-cell adenoma																							
C-cell carcinoma												X											
Parathyroid	A	–	–	–	–	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–
REPRODUCTIVE SYSTEM																							
Mammary gland	N	+	N	+	+	+	N	+	+	N	+	+	+	N	+	+	+	+	+	+	+	N	+	+	+
Adenoma, NOS						X																	
Adenocarcinoma, NOS																		X					
Fibroadenoma		X		X	X			X		X	X	X			X	X	X	X	X	X		X	X	X
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS							X																		
Adenoma, NOS																		X							
Uterus	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS														X											
Adenocarcinoma, NOS			X		X																				
Endometrial stromal polyp		X	X			X			X					X					X					X
Endometrial stromal sarcoma															X										
Fibroadenoma																									
Ovary	A	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																							
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma																					X				

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: : No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	0226	0227	0228	0229	0230	0231	0232	0233	0234	0235	0236	0237	0238	0239	0240	0241	0242	0243	0244	0245	0246	0247	0248	0249	0250	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	0994	0930	0778	1055	0770	0898	0998	1055	0994	0933	0887	0860	0933	0997	0863	0959	0969	1055	1055	0955	0667	0885	0999	1101	1101	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell carcinoma											X															1
Basal cell carcinoma																								X		1
Subcutaneous tissue	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																								X		2
Fibroma							X				X					X		X								10
Carcinosarcoma																	X									1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, NOS, metastatic																						X				1
Sarcoma, NOS, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lipoma																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	–	+	–	–	+	+	–	–	+	+	–	+	–	–	–	+	+	–	–	+	–	+	+	33
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule						X																X				3
Hepatocellular carcinoma																		X								1
Endometrial stromal sarcoma, metasta																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma, NOS																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS																										1
Adenoma, NOS						X	X						X	X				X	X	X	X	X	X	X	X	21
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cortical adenoma																										2
Pheochromocytoma											X															1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma									X									X						X		4
Follicular cell carcinoma																			X							2
C-cell adenoma														X												1
C-cell carcinoma																										1
Parathyroid	–	+	–	–	–	–	+	–	–	+	–	–	–	–	–	+	–	+	–	–	–	+	–	–	–	9
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	+	+	N	+	+	+	+	+	N	+	+	N	N	+	+	+	+	+	N	+	+	+	+	*50
Adenoma, NOS																										1
Adenocarcinoma, NOS						X													X			X		X		5
Fibroadenoma			X	X			X	X	X	X			X			X		X	X	X				X	X	29
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS												X														2
Adenoma, NOS																		X						X		3
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS																										1
Adenocarcinoma, NOS																										2
Endometrial stromal polyp						X						X					X									10
Endometrial stromal sarcoma	X				X													X		X				X		6
Fibroadenoma																									X	1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma																										1

*Animals, Necropsied

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: HIGH DOSE (Continued)

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	083	104	104	104	104	082	082	082	104	083	102	087	105	101	103	092	105	104	105	097	092	087	096	092	105
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	+	N	+	N	N	N	N	N	N	N	+	N	N	N
Carcinoma, NOS												X		X								X			
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Endometrial stromal sarcoma, invasive																X									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS, uncl prim or metastatic																									
Malig. lymphoma, histiocytic type																									
Leukemia, mononuclear cell							X																		

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	094	030	078	105	070	089	098	105	094	093	087	060	093	097	063	095	069	102	105	095	067	085	099	101	101	
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	+	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	+	N	N	N	+	N	N	*50
Carcinoma, NOS			X												X				X				X			7
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Endometrial stromal sarcoma, invasive					X																					2
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS, uns prim or metastatic																X										1
Malig. lymphoma, histiocytic type	X																									1
Leukemia, mononuclear cell				X		X						X	X					X								6

*Animals Necropsied

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(50)	(50)	(50)
ADENOMATOUS POLYP, NOS	1 (2%)		
#LUNG	(50)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST		5 (10%)	12 (24%)
ALVEOLAR/BRONCHIOLAR ADENOMA	7 (14%)	3 (6%)	6 (12%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	4 (8%)	4 (8%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIG. LYMPHOMA, UNDIFFER-TYPE	1 (2%)		
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE	3 (6%)	1 (2%)	4 (8%)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE	2 (4%)	5 (10%)	3 (6%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	3 (6%)
#BONE MARROW	(49)	(47)	(47)
MAST-CELL TUMOR			1 (2%)
#SPLEEN	(49)	(50)	(50)
MALIG. LYMPHOMA, UNDIFFER-TYPE	1 (2%)		
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#LYMPH NODE	(48)	(45)	(47)
SARCOMA, NOS, METASTATIC			1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#MESENTERIC L. NODE	(48)	(45)	(47)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
CIRCULATORY SYSTEM			
#SPLEEN	(49)	(50)	(50)
HEMANGIOMA		1 (2%)	
ANGIOSARCOMA	1 (2%)	1 (2%)	
#LIVER	(50)	(50)	(50)
ANGIOSARCOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
HEPATOCELLULAR ADENOMA	22 (44%)	21 (42%)	17 (34%)
HEPATOCELLULAR CARCINOMA	10 (20%)	20 (40%)	27 (54%)
SARCOMA, NOS, METASTATIC			1 (2%)
MIXED MESENCHYMAL TUMOR, MALIG		1 (2%)	
#STOMACH	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
#DUODENUM	(50)	(48)	(47)
ADENOCARCINOMA, NOS		1 (2%)	2 (4%)
#COLON	(49)	(47)	(47)
CARCINOID TUMOR, NOS	1 (2%)		

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(47)	(48)
ADENOMA, NOS	1 (2%)	2 (4%)	2 (4%)
#ADRENAL	(50)	(50)	(50)
CORTICAL ADENOMA	1 (2%)		1 (2%)
PHEOCHROMOCYTOMA		2 (4%)	2 (4%)
#ADRENAL/CAPSULE	(50)	(50)	(50)
ADENOMA, NOS	2 (4%)		
#THYROID	(50)	(48)	(49)
FOLLICULAR-CELL ADENOMA	1 (2%)	1 (2%)	3 (6%)
REPRODUCTIVE SYSTEM			
#TESTIS	(49)	(48)	(49)
SERTOLI-CELL TUMOR	1 (2%)		
INTERSTITIAL-CELL TUMOR	1 (2%)	1 (2%)	1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS	3 (6%)	5 (10%)	3 (6%)
CYSTADENOMA, NOS	1 (2%)	1 (2%)	1 (2%)
*ZYMBAL GLAND	(50)	(50)	(50)
CARCINOMA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
SARCOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH	6	13	7
MORIBUND SACRIFICE	2	6	8
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	42	31	35
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	44	43	46
TOTAL PRIMARY TUMORS	66	74	81
TOTAL ANIMALS WITH BENIGN TUMORS	32	30	27
TOTAL BENIGN TUMORS	41	38	36
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	32	37
TOTAL MALIGNANT TUMORS	24	36	44
TOTAL ANIMALS WITH SECONDARY TUMORS##		5	12
TOTAL SECONDARY TUMORS		5	15
TOTAL ANIMALS WITH TUMORS UNCERTAIN-- BENIGN OR MALIGNANT	1		1
TOTAL UNCERTAIN TUMORS	1		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* NUMBER OF ANIMALS NECROPSIED
** PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
BASAL-CELL CARCINOMA		1 (2%)	1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS	3 (6%)	2 (4%)	
FIBROMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(47)
CARCINOMA, NOS, METASTATIC	2 (4%)		
BASAL-CELL CARCINOMA, METASTATIC			1 (2%)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR CARCINOMA, METAST		3 (6%)	13 (28%)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	4 (9%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
SARCOMA, NOS, METASTATIC		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	6 (12%)	14 (28%)	11 (22%)
MALIG. LYMPHOMA, UNDIFFER-TYPE	6 (12%)	1 (2%)	1 (2%)
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE		5 (10%)	1 (2%)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	3 (6%)	8 (16%)
MALIGNANT LYMPHOMA, MIXED TYPE	2 (4%)		
#SPLEEN	(49)	(47)	(46)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
#LYMPH NODE	(46)	(46)	(40)
HEPATOCELLULAR CARCINOMA, METAST			1 (3%)
SARCOMA, NOS, METASTATIC	1 (2%)		
MALIGNANT LYMPHOMA, MIXED TYPE			1 (3%)
#LIVER	(49)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)		1 (2%)
#PEYER'S PATCH	(46)	(38)	(43)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#KIDNEY	(50)	(50)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
#SPLEEN	(49)	(47)	(46)
HEMANGIOMA	1 (2%)	1 (2%)	
ANGIOSARCOMA	2 (4%)		
#LYMPH NODE	(46)	(46)	(40)
HEMANGIOMA			1 (3%)
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(49)
NEOPLASM, NOS		1 (2%)	
HEPATOCELLULAR ADENOMA	2 (4%)	18 (36%)	4 (8%)
HEPATOCELLULAR CARCINOMA	3 (6%)	19 (38%)	37 (76%)

TABLE B2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM (Continued)			
#PANCREATIC DUCT	(46)	(45)	(38)
CARCINOMA, NOS	1 (2%)		
#STOMACH	(48)	(46)	(46)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SARCOMA, NOS, INVASIVE	1 (2%)		
#COLON	(45)	(40)	(37)
LEIOMYOSARCOMA	1 (2%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(38)	(36)	(29)
ADENOMA, NOS	10 (26%)	7 (19%)	2 (7%)
#ADRENAL	(48)	(47)	(45)
NEOPLASM, NOS	1 (2%)		
PHEOCHROMOCYTOMA	1 (2%)	7 (15%)	7 (16%)
PHEOCHROMOCYTOMA, MALIGNANT		1 (2%)	1 (2%)
#ADRENAL/CAPSULE	(48)	(47)	(45)
ADENOMA, NOS		2 (4%)	
#THYROID	(45)	(41)	(35)
FOLLICULAR-CELL ADENOMA			2 (6%)
FOLLICULAR-CELL CARCINOMA		2 (5%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
#UTERUS	(50)	(50)	(43)
ADENOCARCINOMA, NOS			1 (2%)
SARCOMA, NOS		1 (2%)	2 (5%)
FIBROMA			1 (2%)
ENDOMETRIAL STROMAL POLYP		1 (2%)	
ENDOMETRIAL STROMAL SARCOMA	1 (2%)		
#OVARY	(48)	(46)	(41)
PAPILLARY CYSTADENOMA, NOS	1 (2%)		
GRANULOSA-CELL TUMOR		1 (2%)	
TUBULAR ADENOMA		1 (2%)	
SARCOMA, NOS, INVASIVE	1 (2%)		
TERATOMA, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
CARCINOMA, NOS	1 (2%)		
ADENOMA, NOS			3 (6%)
CYSTADENOMA, NOS			2 (4%)

TABLE B2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9, MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
CARCINOMA, NOS, INVASIVE	1 (2%)		
SQUAMOUS CELL CARCINOMA, INVASIVE		1 (2%)	
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	19	29	37
MORIBUND SACRIFICE		9	7
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	31	12	6
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	31	50	49
TOTAL PRIMARY TUMORS	45	94	96
TOTAL ANIMALS WITH BENIGN TUMORS	12	27	17
TOTAL BENIGN TUMORS	15	40	26
TOTAL ANIMALS WITH MALIGNANT TUMORS	26	38	47
TOTAL MALIGNANT TUMORS	29	52	69
TOTAL ANIMALS WITH SECONDARY TUMORS##	3	6	15
TOTAL SECONDARY TUMORS	6	7	17
TOTAL ANIMALS WITH TUMORS UNCERTAIN-- BENIGN OR MALIGNANT	1	2	1
TOTAL UNCERTAIN TUMORS	1	2	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* NUMBER OF ANIMALS NECROPSIED
** PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: UNTREATED CONTROL

ANIMAL NUMBER	50101	50201	50301	50401	50501	50601	50701	50801	50901	51001	51101	51201	51301	51401	51501	51601	51701	51801	51901	52001	52101	52201	52301	52401	52501
WEEKS ON STUDY	105	105	105	105	105	105	092	105	105	105	105	105	091	105	105	105	105	105	105	105	105	105	105	105	105
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenomatous polyp, NOS																					X				
Alveolar/bronchiolar adenoma													X		X										
Alveolar/bronchiolar carcinoma		X	X																					X	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Angiosarcoma																									
Malig. lymphoma, undiffer type																									
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	–	+	+	–	+	+	+	–	–	+	+	–	–	–	+	–	–	–	+	–	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma		X			X		X			X	X	X	X		X				X	X	X				X
Hepatocellular carcinoma	X						X										X			X				X	
Angiosarcoma																X									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	N	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoid tumor, NOS									X																
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	–	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS			X																						
Cortical adenoma													X												
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma									X																
Parathyroid	–	–	–	+	+	+	–	+	–	+	+	–	–	+	+	–	–	–	+	+	+	+	+	–	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sertoli cell tumor																									
Interstitial cell tumor																									
Prostate	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS			X													X						X			
Cystadenoma, NOS																									
Zymbal gland	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS								X																	
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malig. lymphoma, undiffer type											X														
Malig. lymphoma, lymphocytic type																									
Malig. lymphoma, histiocytic type				X																					

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: : No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	5/26	5/27	5/28	5/29	5/30	5/31	5/32	5/33	5/34	5/35	5/36	5/37	5/38	5/39	5/40	5/41	5/42	5/43	5/44	5/45	5/46	5/47	5/48	5/49	5/50	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	10/5	10/5	10/5	10/5	10/5	10/5	10/3	09/1	10/5	10/5	10/5	09/6	08/4	10/5	10/5	09/7	10/5	10/5	10/5	10/5	10/5	10/5	09/8	10/5	10/5	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenomatous polyp, NOS							X																			1
Alveolar/bronchiolar adenoma					X	X	X																			7
Alveolar/bronchiolar carcinoma			X																						X	4
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenomatous polyp, NOS							X																			1
Alveolar/bronchiolar adenoma					X	X	X																			7
Alveolar/bronchiolar carcinoma			X																						X	4
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Angiosarcoma							X																			1
Malig. lymphoma, undiffer type		X																								1
Lymph nodes	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	–	–	–	–	–	–	–	+	+	–	–	+	+	–	–	–	–	+	–	+	–	24
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma	X			X	X		X		X	X	X	X				X							X			22
Hepatocellular carcinoma		X								X					X									X		X	10
Angiosarcoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	N	+	+	+	+	+	N	+	+	N	+	+	+	+	+	+	N	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	49
Carcinoid tumor, NOS																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoma, NOS													X													1
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS																	X									2
Cortical adenoma																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma																										1
Parathyroid	–	–	–	–	–	+	–	+	–	–	–	–	+	–	+	+	–	+	+	+	+	+	+	+	+	27
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sertoli cell tumor																		X								1
Interstitial cell tumor																		X								1
Prostate	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS																										3
Cystadenoma, NOS															X											1
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malig. lymphoma, undiffer type																										1
Malig. lymphoma, lymphocytic type													X					X	X							3
Malig. lymphoma, histiocytic type								X																		2

* Animals Necropsied

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: LOW DOSE

ANIMAL NUMBER	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525
WEEKS ON STUDY	105	078	105	105	088	105	105	062	105	096	105	105	073	073	094	105	105	076	105	079	001	089	105	105	105
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic										X										X					
Alveolar/bronchiolar adenoma			X			X																			
Alveolar/bronchiolar carcinoma																									X
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Angiosarcoma																									
Malignant lymphoma, mixed type																			X						
Lymph nodes	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Malignant lymphoma, mixed type																									
Thymus	+	–	+	+	–	–	+	–	–	–	+	–	+	–	+	–	+	–	+	–	–	–	–	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma					X	X		X								X	X					X	X		X
Hepatocellular carcinoma		X	X		X		X		X	X				X					X	X				X	
Mixed mesenchymal tumor, malignant											X														
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	+	N	+	+	N	+	N	+	+	N	N	+	+	+	+	+	N	N	N	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Small intestine	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
Adenocarcinoma, NOS																		X							
Large intestine	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS									X																
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma											X			X											
Thyroid	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																				X					
Parathyroid	+	+	+	–	+	–	–	–	–	+	+	+	–	+	–	–	+	–	+	–	+	+	–	+	–
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	
Interstitial cell tumor												X													
Prostate	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	+	+	+	–	+	
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS											X		X												
Cystadenoma, NOS																			X						
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malig. lymphoma, lymphocytic type																						X			
Malig. lymphoma, histiocytic type																									
Malignant lymphoma, mixed type											X														

\+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	52 6	52 7	52 8	52 9	53 0	53 1	53 2	53 3	53 4	53 5	53 6	53 7	53 8	53 9	54 0	54 1	54 2	54 3	54 4	54 5	54 6	54 7	54 8	54 9	55 0	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	10 5	10 5	10 5	10 5	10 5	10 5	10 5	10 5	10 5	10 5	10 5	10 5	10 5	09 4	09 8	10 5	09 2	10 5	10 5	10 5	10 5	09 8	07 2	07 6	10 4	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic									X						X	X										5
Alveolar/bronchiolar adenoma														X												3
Alveolar/bronchiolar carcinoma	X										X											X				4
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma											X															1
Angiosarcoma																X										1
Malignant lymphoma, mixed type																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+	+	+	+	45
Malignant lymphoma, mixed type								X																		1
Thymus	+	–	–	–	+	+	+	+	+	–	–	+	–	–	–	+	–	+	+	+	+	–	+	+	–	25
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma		X		X	X	X				X	X			X		X		X	X	X	X			X		21
Hepatocellular carcinoma			X	X			X		X						X	X		X					X	X	X	20
Mixed mesenchymal tumor, malignant																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	N	+	+	+	+	N	N	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma			X																							1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenocarcinoma, NOS																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	47
Adenoma, NOS								X																		2
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma																										2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell adenoma																										1
Parathyroid	+	–	+	+	+	+	+	–	–	–	–	+	–	+	+	–	–	+	+	+	+	+	+	–	+	29
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Interstitial cell tumor																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS								X	X												X					5
Cystadenoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malig. lymphoma, lymphocytic type														X												1
Malig. lymphoma, histiocytic type										X					X									X	X	5
Malignant lymphoma, mixed type																										1

* Animals Necropsied

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF C. I. BASIC RED 9 MONOHYDROCHLORIDE: HIGH DOSE

ANIMAL NUMBER	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525
WEEKS ON STUDY	104	049	104	104	104	104	104	075	104	104	104	104	105	079	095	105	083	104	105	105	105	105	105	105	105
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma												X													
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic			X					X							X	X	X		X			X			
Alveolar/bronchiolar adenoma											X						X								
Alveolar/bronchiolar carcinoma			X																						
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS, metastatic																X									
Malignant lymphoma, mixed type																									
Thymus	+	–	–	+	+	+	–	–	–	+	+	–	+	–	–	+	+	–	–	+	+	–	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma	X			X		X													X				X		X
Hepatocellular carcinoma			X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X				
Sarcoma, NOS, metastatic																X									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	N	+	+	+	+	N	+	N	N	N	N	N	+	N	+	+	N	+	N	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Large intestine	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	X	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic																			X						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																									
Pheochromocytoma			X																						
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma			X						X																
Parathyroid	–	–	–	–	+	+	–	+	+	+	+	+	–	–	–	–	–	–	+	–	+	–	+	–	–
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
Interstitial cell tumor																									
Prostate	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
Cystadenoma, NOS												X													
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS																X									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malig. lymphoma, lymphocytic type						X																			
Malig. lymphoma, histiocytic type																			X						
Malignant lymphoma, mixed type								X															X		

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (*Continued*)

ANIMAL NUMBER	52 26	52 27	52 28	52 29	53 30	53 31	53 32	53 33	53 34	53 35	53 36	53 37	53 38	53 39	54 40	54 41	54 42	54 43	54 44	54 45	54 46	54 47	54 48	54 49	55 50	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	08 96	08 95	10 05	10 91	09 05	10 05	10 05	10 05	01 06	06 55	10 00	10 05	09 98	10 05	10 05	10 05	10 05	10 05	10 00	06 06	10 05	10 05	10 05	10 05	05 50	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell carcinoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic	X				X													X					X	X		12
Alveolar/bronchiolar adenoma			X					X									X					X			6
Alveolar/bronchiolar carcinoma							A																			2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	47
Mast cell tumor																X										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	47
Sarcoma, NOS, metastatic																										1
Malignant lymphoma, mixed type																			X							1
Thymus	–	–	+	+	–	+	+	+	+	–	+	+	–	+	+	+	+	–	–	+	+	–	–	–	+	29
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma			X	X		X	X	X			X		X	X		X					X			X		17
Hepatocellular carcinoma	X	X		X	X							X					X	X		X			X	X		27
Sarcoma, NOS, metastatic																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	N	+	+	N	+	N	+	+	+	+	N	+	+	+	+	+	N	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–	+	+	+	+	45
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	47
Adenocarcinoma, NOS	X				X																					2
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS		X												X												2
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cortical adenoma				X																						1
Pheochromocytoma			X																							2
Thyroid	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell adenoma					X																					3
Parathyroid	+	–	–	–	+	+	–	+	–	–	+	+	+	+	–	–	–	–	–	–	–	+	+	+	+	22
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Interstitial cell tumor																					X					1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	46
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS			X																	X			X			3
Cystadenoma, NOS																										1
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malig. lymphoma, lymphocytic type									X													X			X	4
Malig. lymphoma, histiocytic type										X			X													3
Malignant lymphoma, mixed type											X															3

*Animals Necropsied

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C. I. BASIC RED 9 MONOHYDROCHLORIDE: UNTREATED CONTROL

ANIMAL NUMBER	0091	0092	0093	0094	0095	0096	0097	0098	0099	0100	0111	0112	0113	0114	0115	0116	0117	0118	0119	0120	0121	0122	0123	0124	0125
WEEKS ON STUDY	105	101	105	105	084	095	105	105	105	084	075	105	098	084	072	105	105	105	105	105	096	085	098	105	105
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	N	+	+	+	N	N	+	+	+	+	+	+	+	+	+	+	N	+	+	+
Sarcoma, NOS		X																							
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, metastatic					X						X														
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	–	+	+	+	+	+	+	–	+	+	+	+	+	–	–	+	+	+	+	–	–	+	+
Spleen	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Angiosarcoma																									X
Lymph nodes	+	+	+	+	–	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS, metastatic		X																							
Thymus	+	–	–	+	–	–	+	+	+	–	–	+	–	–	–	+	–	+	–	+	+	–	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma				X																					
Hepatocellular carcinoma																									
Malignant lymphoma, NOS																		X							
Bile duct	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	N	N	+	+	N	N	N	+	+	+	N	+	+	+	+	+	+	+	N	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS											X														
Esophagus	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Sarcoma, NOS, invasive		X																							
Small intestine	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Malignant lymphoma, NOS																									
Large intestine	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Leiomyosarcoma																							X		
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	–	+	–	+	–	–	+	–	+	+	+	–	+	–	+	+	+	–	–	+	–	+	+
Adenoma, NOS		X											X											X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
Neoplasm, NOS										X															
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	–	+	–	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+
Parathyroid	+	–	+	+	–	+	–	–	–	–	–	+	+	–	+	+	–	+	+	+	+	–	–	–	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal sarcoma									X																
Ovary	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papillary cystadenoma, NOS																									
Sarcoma, NOS, invasive		X																							
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS					X																				
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS, invasive											X														
Malignant lymphoma, NOS																					X				
Malig. lymphoma, undiffer type							X						X										X		
Malig. lymphoma, histiocytic type														X											
Malignant lymphoma, mixed type																									

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	0226	0227	0228	0229	0330	0331	0332	0333	0334	0335	0336	0337	0338	0339	0340	0341	0342	0343	0344	0345	0346	0347	0348	0349	0350	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	105	105	045	058	088	105	105	097	088	101	105	105	105	105	105	105	091	105	111	099	095	105	105	083	105	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS				X													X									3
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, metastatic																										2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	−	−	+	+	+	+	+	−	+	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	38
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangioma					X																					1
Angiosarcoma										X																2
Lymph nodes	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Sarcoma, NOS, metastatic																										1
Thymus	+	+	−	+	−	+	+	+	−	+	+	+	+	+	−	+	+	−	+	+	−	+	+	−	+	31
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma															X											2
Hepatocellular carcinoma		X								X				X												3
Malignant lymphoma, NOS																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	N	+	N	+	N	+	+	+	N	+	N	+	N	+	+	N	+	N	+	+	+	+	+	N	+	*50
Pancreas	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+		46
Carcinoma, NOS																										1
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+		47
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Sarcoma, NOS, invasive																										1
Small intestine	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		46
Malignant lymphoma, NOS																X										1
Large intestine	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		45
Leiomyosarcoma																										1
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+			38
Adenoma, NOS					X					X				X	X	X		X					X			10
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+			48
Neoplasm, NOS																										1
Pheochromocytoma											X															1
Thyroid	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+			45
Parathyroid	+	+	+	+	−	−	+	−	−	−	+	−	+	+	+	−	−	+	+	−	+	−	+			28
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	+	N	N	N	N	N	N	N	N	+	N	+	N	N	N	N	N	N	N	N			*50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Endometrial stromal sarcoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+			48
Papillary cystadenoma, NOS										X																1
Sarcoma, NOS, invasive																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N			*50
Carcinoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N			*50
Carcinoma, NOS, invasive																										1
Malignant lymphoma, NOS				X									X				X	X					X			6
Malig. lymphoma, undiffer type					X						X								X							6
Malig. lymphoma, histiocytic type																										1
Malignant lymphoma, mixed type	X																				X					2

* Animals Necropsied

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I BASIC RED 9 MONOHYDROCHLORIDE: LOW DOSE

ANIMAL NUMBER	0001	0002	0003	0004	0005	0006	0007	0008	0009	0010	0011	0012	0013	0014	0015	0016	0017	0018	0019	0020	0021	0022	0023	0024	0025
WEEKS ON STUDY	105	059	079	051	061	093	104	082	085	081	081	055	034	044	057	011	099	091	060	048	073	105	085	081	093
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	N	+
Basal cell carcinoma																	X								
Subcutaneous tissue	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	N	+
Sarcoma, NOS																									
Fibroma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS, metastatic										X															
Hepatocellular carcinoma, metastatic								X									X								
Alveolar/bronchiolar adenoma			X																						
Sarcoma, NOS, metastatic																									
Trachea	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	–	–	+	+	+	+	+	+
Spleen	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Malignant lymphoma, NOS	X																								
Lymph nodes	+	+	+	+	+	–	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Thymus	+	–	–	+	–	–	–	–	+	–	+	+	+	–	–	+	+	–	–	–	–	+	–	–	–
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	–	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplasm, NOS																									
Hepatocellular adenoma			X	X							X			X		X			X			X		X	X
Hepatocellular carcinoma	X				X	X	X	X	X	X								X	X				X			
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	N	+	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	+	N	+	+
Pancreas	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	–	–	–	+	+	+	+	+	–	+	+	–	+	+	–	+	+	+	+	+	+
Stomach	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Small intestine	+	+	+	+	+	–	–	+	+	+	+	–	–	+	+	+	+	–	+	+	–	+	+	–	+
Large intestine	+	+	+	+	+	+	–	+	+	+	+	+	–	+	–	+	+	–	+	+	–	+	+	–	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	–	+	+	–	–	+	+	–	+	–	+	+	–	+	+	+	+	+	+	+	+	+	–
Adenoma, NOS																X									
Adrenal	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Adenoma, NOS																									
Pheochromocytoma	X						X														X				
Pheochromocytoma, malignant																									
Thyroid	+	+	+	+	+	–	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma	X																								
Parathyroid	–	–	–	+	+	–	–	–	–	–	+	–	–	–	+	–	–	+	–	+	+	–	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N
Adenocarcinoma, NOS										X															
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS									X																
Endometrial stromal polyp											X														
Ovary	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor								X																	
Tubular adenoma		X																							
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	–	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma, invasive																									
Squamous cell carcinoma, metastatic																									
Malignant lymphoma, NOS		X	X						X			X	X	X	X		X			X	X				
Malig. lymphoma, undiffer type																									
Malig. lymphoma, lymphocytic type											X					X									
Malig. lymphoma, histiocytic type																						X			

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	105	092	105	089	038	105	105	084	098	092	084	105	088	062	105	050	088	082	090	091	089	105	098	080	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	*50
Basal cell carcinoma																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	*50
Sarcoma, NOS					X																				X	2
Fibroma																					X					1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma, NOS, metastatic																										1
Hepatocellular carcinoma, metastatic																					X					3
Alveolar/bronchiolar adenoma														X												2
Sarcoma, NOS, metastatic																									X	1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	44
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	47
Hemangioma																					X					1
Malignant lymphoma, NOS																										1
Lymph nodes	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Thymus	–	–	–	+	–	+	+	+	+	+	–	–	+	–	–	+	+	–	–	–	–	–	–	+	–	20
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
Salivary gland	+	+	–	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	42
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplasm, NOS																			X							1
Hepatocellular adenoma				X	X		X	X		X		X	X	X		X										18
Hepatocellular carcinoma		X	X					X								X		X			X	X	X	X		19
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	N	+	N	+	N	N	+	+	+	+	N	+	N	+	+	N	+	N	N	+	N	N	+	+	+	*50
Pancreas	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	45
Esophagus	+	+	+	–	–	+	+	–	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	39
Stomach	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	46
Squamous cell carcinoma																					X					1
Small intestine	–	+	+	+	–	–	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	38
Large intestine	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	40
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	–	+	–	+	+	+	+	+	–	+	–	–	+	–	–	+	+	+	+	+	+	+	36
Adenoma, NOS					X			X		X			X			X							X			7
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	47
Adenoma, NOS				X												X										2
Pheochromocytoma						X				X	X		X													7
Pheochromocytoma, malignant	X																									1
Thyroid	+	+	+	+	–	–	+	+	–	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	41
Follicular cell carcinoma											X															2
Parathyroid	–	+	–	+	–	–	+	+	–	–	–	+	+	+	+	–	+	+	–	+	–	+	+	+	–	24
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	+	N	N	N	N	N	+	N	N	N	N	N	N	+	N	N	N	N	+	*50
Adenocarcinoma, NOS																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, NOS																										1
Endometrial stromal polyp																										1
Ovary	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	46
Granulosa cell tumor																										1
Tubular adenoma																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell carcinoma, invasive																				X						1
Squamous cell carcinoma, metastatic																				X						1
Malignant lymphoma, NOS	X				X												X	X							14
Malig. lymphoma, undiffer type								X																		1
Malig. lymphoma, lymphocytic type									X							X							X			5
Malig. lymphoma, histiocytic type															X					X						3

* Animals Necropsied

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE: HIGH DOSE

ANIMAL NUMBER	0012	0092	0093	0094	0095	0096	0097	0098	0099	0100	0111	0112	0113	0114	0115	0116	0117	0118	0119	0120	0121	0122	0123	0124	0125
WEEKS ON	1020	0611	0777	0771	0885	0837	0893	0955	0866	0905	0852	0852	0852	0654	0924	0901	0711	0611	0955	0843	0986	0689	0231	0534	0665
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+
Basal cell carcinoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Basal cell carcinoma, metastatic																									
Hepatocellular carcinoma, metastatic	X			X		X					X	X			X										
Alveolar/bronchiolar adenoma		X																							
Alveolar/bronchiolar carcinoma																									
Sarcoma, NOS, metastatic																									
Trachea	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, NOS										X															
Lymph nodes	+	–	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+
Hepatocellular carcinoma, metastatic					X																				
Hemangioma					X																				
Malignant lymphoma, mixed type																									
Thymus	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	–	–	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma														X											
Hepatocellular carcinoma	X			X		X	X	X	X	X	X	X	X		X	X	X		X	X	X	X			X
Malignant lymphoma, NOS																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	+	+	N	N	N	N	N	+	N	N	+	N	N	N	+	N	N	N	N	N	N	+
Pancreas	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	–	+	–	+	–	+	+	+
Esophagus	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	–	+	–	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Small intestine	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Large intestine	+	–	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, NOS											X														
Urinary bladder	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	–	–	–	+	+	+	–	–	+	+	+	+	+	+	–	+	–	+	–	+	+	+	–	+
Adenoma, NOS																					X				
Adrenal	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Pheochromocytoma											X	X							X		X				
Pheochromocytoma, malignant																									
Thyroid	+	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	+	+
Follicular cell adenoma											X														
Parathyroid	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–	+	–	–	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	+	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenocarcinoma, NOS								X																	
Uterus	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–
Adenocarcinoma, NOS																									
Sarcoma, NOS				X																					
Fibroma																									
Ovary	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	
Teratoma, NOS																							X		
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS						X																			
Cystadenoma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenocarcinoma, NOS, metastatic																									
Malignant lymphoma, NOS		X																		X		X	X		
Malig. lymphoma, undiffer type																			X						
Malig. lymphoma, lymphocytic type																									
Malig. lymphoma, histiocytic type			X		X									X	X	X		X			X				X

- • : Tissue Examined Microscopically
- – : Required Tissue Not Examined Microscopically
- X : Tumor Incidence
- N : Necropsy, No Autolysis, No Microscopic Examination
- S : Animal Missexed
- : : No Tissue Information Submitted
- C : Necropsy, No Histology Due To Protocol
- A : Autolysis
- M : Animal Missing
- B : No Necropsy Performed

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER / WEEKS ON STUDY / TISSUES	TOTAL: TISSUES TUMORS
SKIN	
Skin	*50
Basal cell carcinoma	1
RESPIRATORY SYSTEM	
Lungs and bronchi	47
Basal cell carcinoma, metastatic	1
Hepatocellular carcinoma, metastatic	13
Alveolar/bronchiolar adenoma	4
Alveolar/bronchiolar carcinoma	1
Sarcoma, NOS, metastatic	1
Trachea	47
HEMATOPOIETIC SYSTEM	
Bone marrow	41
Spleen	46
Malignant lymphoma, NOS	1
Lymph nodes	40
Hepatocellular carcinoma, metastatic	1
Hemangioma	1
Malignant lymphoma, mixed type	1
Thymus	5
CIRCULATORY SYSTEM	
Heart	47
DIGESTIVE SYSTEM	
Salivary gland	45
Liver	49
Hepatocellular adenoma	4
Hepatocellular carcinoma	37
Malignant lymphoma, NOS	1
Bile duct	49
Gallbladder & common bile duct	*50
Pancreas	38
Esophagus	42
Stomach	46
Small intestine	43
Large intestine	37
URINARY SYSTEM	
Kidney	48
Malignant lymphoma, NOS	1
Urinary bladder	45
ENDOCRINE SYSTEM	
Pituitary	29
Adenoma, NOS	2
Adrenal	45
Pheochromocytoma	7
Pheochromocytoma, malignant	1
Thyroid	35
Follicular cell adenoma	2
Parathyroid	18
REPRODUCTIVE SYSTEM	
Mammary gland	*50
Adenocarcinoma, NOS	1
Uterus	43
Adenocarcinoma, NOS	1
Sarcoma, NOS	2
Fibroma	1
Ovary	41
Teratoma, NOS	1
NERVOUS SYSTEM	
Brain	48
SPECIAL SENSE ORGANS	
Harderian gland	*50
Adenoma, NOS	3
Cystadenoma, NOS	2
ALL OTHER SYSTEMS	
Multiple organs, NOS	*50
Adenocarcinoma, NOS, metastatic	1
Malignant lymphoma, NOS	11
Malig. lymphoma, undiffer type	1
Malig. lymphoma, lymphocytic type	1
Malig. lymphoma, histiocytic type	8

* Animals Necropsied

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)	1 (2%)	3 (6%)
INFLAMMATION, NOS	1 (2%)	1 (2%)	7 (14%)
INFLAMMATION, ACUTE	1 (2%)		1 (2%)
NECROSIS, NOS			6 (12%)
HYPERPLASIA, EPITHELIAL	1 (2%)		
HYPERPLASIA, BASAL CELL			5 (10%)
HYPERKERATOSIS	2 (4%)	2 (4%)	10 (20%)
ACANTHOSIS			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
INFLAMMATION, NOS	1 (2%)	1 (2%)	
REACTION, FOREIGN BODY		1 (2%)	
NECROSIS, NOS	1 (2%)	1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(50)	(50)	(50)
INFLAMMATION, NOS		2 (4%)	3 (6%)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		3 (6%)	
NECROSIS, FIBRINOID			1 (2%)
#LUNG/BRONCHUS	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	1 (2%)
INFLAMMATION, FOCAL			2 (4%)
#LUNG/BRONCHIOLE	(50)	(50)	(50)
INFLAMMATION, NOS		2 (4%)	
#LUNG	(50)	(50)	(50)
MINERALIZATION			2 (4%)
HEMORRHAGE		4 (8%)	
BRONCHOPNEUMONIA, NOS			3 (6%)
INFLAMMATION, NOS	6 (12%)	4 (8%)	18 (36%)
INFLAMMATION, FOCAL	2 (4%)	4 (8%)	
INFLAMMATION, ACUTE		3 (6%)	9 (18%)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)	1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOUS			1 (2%)
REACTION, FOREIGN BODY		1 (2%)	1 (2%)
FIBROSIS			1 (2%)
HYPERPLASIA, EPITHELIAL	1 (2%)	3 (6%)	2 (4%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(49)	(49)	(49)
DEGENERATION, LIPOID		1 (2%)	
NECROSIS, FOCAL		1 (2%)	1 (2%)
LYMPHOID DEPLETION	2 (4%)	3 (6%)	9 (18%)
HEMATOPOIESIS	22 (45%)	37 (76%)	28 (57%)
#SPLENIC FOLLICLES	(49)	(49)	(49)
ATROPHY, NOS		1 (2%)	1 (2%)

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#LYMPH NODE	(49)	(49)	(48)
ATROPHY, NOS	1 (2%)		
LYMPHOID DEPLETION			2 (4%)
ANGIECTASIS	1 (2%)	2 (4%)	4 (8%)
PLASMACYTOSIS	2 (4%)		1 (2%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	2 (4%)
HEMATOPOIESIS			1 (2%)
#LIVER	(50)	(50)	(50)
HEMATOPOIESIS		1 (2%)	4 (8%)
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(50)
MINERALIZATION			2 (4%)
THROMBOSIS, NOS	1 (2%)		
INFLAMMATION, FOCAL	1 (2%)		1 (2%)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
FIBROSIS	1 (2%)		1 (2%)
#MYOCARDIUM	(50)	(50)	(50)
DEGENERATION, NOS	46 (92%)	44 (88%)	28 (56%)
*ARTERY	(50)	(50)	(50)
PERIVASCULITIS		1 (2%)	
#PANCREAS	(47)	(50)	(46)
PERIVASCULITIS		1 (2%)	
#STOMACH	(48)	(50)	(48)
PERIARTERITIS			1 (2%)
DIGESTIVE SYSTEM			
*MOUTH	(50)	(50)	(50)
NECROSIS, NOS			1 (2%)
HYPERKERATOSIS			1 (2%)
ACANTHOSIS			1 (2%)
*ORAL MUCOUS MEMBRANE	(50)	(50)	(50)
INFLAMMATION, ACUTE	1 (2%)		
ACANTHOSIS	1 (2%)		
#SALIVARY GLAND	(48)	(47)	(40)
HYPERPLASIA, FOCAL		1 (2%)	
#LIVER	(50)	(50)	(50)
CONGENITAL MALFORMATION, NOS	1 (2%)		
INFLAMMATION, NOS			1 (2%)
DEGENERATION, NOS	2 (4%)	1 (2%)	
NECROSIS, FOCAL		3 (6%)	8 (16%)
NECROSIS, ISCHEMIC	1 (2%)	1 (2%)	10 (20%)
METAMORPHOSIS FATTY	20 (40%)	33 (66%)	23 (46%)
CYTOPLASMIC CHANGE, NOS			1 (2%)
BASOPHILIC CYTO CHANGE	6 (12%)		2 (4%)
FOCAL CELLULAR CHANGE	30 (60%)	41 (82%)	29 (58%)
CLEAR-CELL CHANGE	2 (4%)		
REGENERATION, NOS	1 (2%)		
#PORTAL TRACT	(50)	(50)	(50)
FIBROSIS			1 (2%)
#LIVER/CENTRILOBULAR	(50)	(50)	(50)
NECROSIS, NOS	1 (2%)		2 (4%)
#BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS	33 (66%)	13 (26%)	3 (6%)
#PANCREAS	(47)	(50)	(46)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM (Continued)			
#PANCREATIC ACINUS	(47)	(50)	(46)
ATROPHY, NOS	1 (2%)	3 (6%)	
ATROPHY, FOCAL		2 (4%)	
HYPERTROPHY, FOCAL			2 (4%)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	1 (2%)		1 (2%)
#ESOPHAGUS	(45)	(41)	(44)
HEMORRHAGE			1 (2%)
HYPERKERATOSIS	2 (4%)	1 (2%)	2 (5%)
#STOMACH	(48)	(50)	(48)
MINERALIZATION		1 (2%)	1 (2%)
EDEMA, NOS	1 (2%)		
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS	1 (2%)		2 (4%)
ULCER, NOS	2 (4%)		
INFLAMMATION, ACUTE		1 (2%)	
ABSCESS, NOS	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)	2 (4%)	
FIBROSIS	1 (2%)		
NECROSIS, NOS	1 (2%)	1 (2%)	
NECROSIS, FOCAL		3 (6%)	2 (4%)
HYPERPLASIA, EPITHELIAL	1 (2%)	3 (6%)	10 (21%)
HYPERPLASIA, BASAL CELL		3 (6%)	2 (4%)
HYPERKERATOSIS	4 (8%)	5 (10%)	10 (21%)
ACANTHOSIS			2 (4%)
#GASTRIC SUBMUCOSA	(48)	(50)	(48)
INFLAMMATION, NOS	1 (2%)	1 (2%)	
INFLAMMATION, ACUTE	1 (2%)		
#FORESTOMACH	(48)	(50)	(48)
HYPERPLASIA, EPITHELIAL			1 (2%)
#PEYER'S PATCH	(44)	(48)	(42)
HYPERPLASIA, NOS	4 (9%)	6 (13%)	5 (12%)
#DUODENUM	(44)	(48)	(42)
FIBROSIS			1 (2%)
NECROSIS, NOS			1 (2%)
#JEJUNUM AND ILEUM COMBINED SITE	(44)	(48)	(42)
INFLAMMATION, NOS	1 (2%)		
NECROSIS, NOS	1 (2%)		
#COLON	(45)	(45)	(42)
HEMORRHAGE			1 (2%)
PARASITISM	6 (13%)	2 (4%)	1 (2%)
NECROSIS, NOS			1 (2%)
#CECUM	(45)	(45)	(42)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
FIBROSIS		1 (2%)	
NECROSIS, NOS		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(49)
MINERALIZATION		2 (4%)	6 (12%)
HYDRONEPHROSIS			1 (2%)
HEMORRHAGE		2 (4%)	
INFLAMMATION, NOS	13 (27%)	26 (52%)	6 (12%)
INFLAMMATION, ACUTE/CHRONIC			2 (4%)
INFLAMMATION, CHRONIC	1 (2%)		
FIBROSIS, DIFFUSE	8 (16%)	19 (38%)	11 (22%)
NEPHROPATHY	48 (98%)	47 (94%)	44 (90%)
NECROSIS, FOCAL	1 (2%)		

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
URINARY SYSTEM (Continued)			
#RENAL PAPILLA	(49)	(50)	(49)
MINERALIZATION		1 (2%)	
NECROSIS, NOS			1 (2%)
#KIDNEY/TUBULE	(49)	(50)	(49)
MINERALIZATION			1 (2%)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL	1 (2%)	1 (2%)	
#URINARY BLADDER	(47)	(49)	(47)
INFLAMMATION, NOS	1 (2%)		
HYPERPLASIA, EPITHELIAL	1 (2%)		
#U.BLADDER/SUBMUCOSA	(47)	(49)	(47)
HEMORRHAGE			2 (4%)
NECROSIS, NOS			2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(47)	(46)
DILATATION, NOS	4 (8%)	2 (4%)	1 (2%)
HYPERPLASIA, NOS	1 (2%)	3 (6%)	
HYPERPLASIA, FOCAL	2 (4%)	1 (2%)	1 (2%)
#ADRENAL	(49)	(49)	(48)
MINERALIZATION	1 (2%)		
DILATATION, NOS		1 (2%)	3 (6%)
INFLAMMATION, NOS	1 (2%)		
NECROSIS, NOS	3 (6%)		
METAMORPHOSIS FATTY	3 (6%)	2 (4%)	3 (6%)
#ADRENAL CORTEX	(49)	(49)	(48)
HYPERTROPHY, FOCAL	3 (6%)	1 (2%)	2 (4%)
#ADRENAL MEDULLA	(49)	(49)	(48)
HYPERPLASIA, NOS	8 (16%)	5 (10%)	6 (13%)
HYPERPLASIA, FOCAL	2 (4%)	1 (2%)	2 (4%)
#THYROID	(49)	(46)	(44)
MINERALIZATION			1 (2%)
FOLLICULAR CYST, NOS		3 (7%)	3 (7%)
INFLAMMATION, NOS			1 (2%)
FIBROSIS			1 (2%)
NECROSIS, NOS			1 (2%)
HYPERPLASIA, C-CELL	2 (4%)	2 (4%)	
HYPERPLASIA, FOLLICULAR-CELL			16 (36%)
METAPLASIA, SQUAMOUS			1 (2%)
#PANCREATIC ISLETS	(47)	(50)	(46)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
GALACTOCELE		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
*PREPUTIAL GLAND	(50)	(50)	(50)
INFLAMMATION, NOS	2 (4%)	1 (2%)	
NECROSIS, NOS	3 (6%)	1 (2%)	
HYPERPLASIA, NOS	2 (4%)		
#PROSTATE	(48)	(48)	(48)
INFLAMMATION, NOS	17 (35%)	19 (40%)	10 (21%)
INFLAMMATION, ACUTE FOCAL			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
HYPERPLASIA, FOCAL	1 (2%)	2 (4%)	1 (2%)

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9, MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCIVE SYSTEM (Continued)			
#TESTIS	(48)	(48)	(50)
MINERALIZATION	5 (10%)	10 (21%)	11 (22%)
ATROPHY, NOS	32 (67%)	34 (71%)	23 (46%)
HYPERPLASIA, INTERSTITIAL CELL	4 (8%)	2 (4%)	8 (16%)
#TESTIS/TUBULE	(48)	(48)	(50)
ATROPHY, FOCAL			4 (8%)
NERVOUS SYSTEM			
#BRAIN	(49)	(50)	(50)
HYDROCEPHALUS, NOS	1 (2%)		
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
CATARACT			1 (2%)
*EXTERNAL EAR	(50)	(50)	(50)
HYPERKERATOSIS			1 (2%)
*ZYMBAL GLAND	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	1 (2%)
NECROSIS, NOS		1 (2%)	6 (12%)
HYPERKERATOSIS		1 (2%)	8 (16%)
MUSCULOSKELETAL SYSTEM			
*PHALANGES	(50)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
FIBROSIS	1 (2%)		
OSTEOARTHRITIS	1 (2%)		
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
METAPLASIA, OSSEOUS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS			3 (6%)
DEGENERATION, NOS			1 (2%)
NECROSIS, NOS			2 (4%)
ATROPHY, NOS			1 (2%)
OMENTUM			
INFLAMMATION, ACUTE/CHRONIC	1		
NECROSIS, FAT	1	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 IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)	1 (2%)	
INFLAMMATION, NOS	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, ACUTE			1 (2%)
FIBROSIS			1 (2%)
NECROSIS, NOS		2 (4%)	2 (4%)
METAPLASIA, OSSEOUS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	1 (2%)
#LUNG	(50)	(50)	(50)
MINERALIZATION	1 (2%)		
HEMORRHAGE		3 (6%)	
INFLAMMATION, NOS	1 (2%)	10 (20%)	6 (12%)
INFLAMMATION, FOCAL	7 (14%)	1 (2%)	
INFLAMMATION, ACUTE		4 (8%)	5 (10%)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION, GRANULOMATOUS			1 (2%)
HYPERPLASIA, EPITHELIAL	3 (6%)	3 (6%)	3 (6%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(49)	(49)	(50)
FIBROSIS		1 (2%)	
LYMPHOID DEPLETION		3 (6%)	5 (10%)
HEMATOPOIESIS	36 (73%)	39 (80%)	35 (70%)
#SPLENIC FOLLICLES	(49)	(49)	(50)
ATROPHY, NOS		8 (16%)	4 (8%)
#LYMPH NODE	(50)	(49)	(49)
INFLAMMATION, NOS		1 (2%)	
ABSCESS, NOS		1 (2%)	
NECROSIS, NOS		1 (2%)	
ANGIECTASIS		1 (2%)	1 (2%)
PLASMACYTOSIS		4 (8%)	1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)		2 (4%)
HEMATOPOIESIS	1 (2%)		
#LIVER	(50)	(50)	(50)
HEMATOPOIESIS		1 (2%)	2 (4%)
#ADRENAL	(50)	(50)	(49)
HEMATOPOIESIS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(50)
MINERALIZATION	1 (2%)		2 (4%)
THROMBUS, MURAL		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	1 (2%)	1 (2%)	
#MYOCARDIUM	(50)	(50)	(50)
DEGENERATION, NOS	38 (76%)	34 (68%)	30 (60%)

TABLE C2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
CONGENITAL MALFORMATION, NOS	1 (2%)		
DILATATION, NOS		1 (2%)	
CONGESTION, NOS		1 (2%)	
DEGENERATION, NOS		3 (6%)	2 (4%)
NECROSIS, FOCAL	4 (8%)	5 (10%)	4 (8%)
NECROSIS, ISCHEMIC	1 (2%)	3 (6%)	3 (6%)
METAMORPHOSIS FATTY	15 (30%)	24 (48%)	16 (32%)
BASOPHILIC CYTO CHANGE	18 (36%)	11 (22%)	8 (16%)
FOCAL CELLULAR CHANGE	28 (56%)	33 (66%)	31 (62%)
REGENERATION, NOS		1 (2%)	
#BILE DUCT	(50)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
FIBROSIS		1 (2%)	
SCLEROSIS		1 (2%)	1 (2%)
HYPERPLASIA, NOS	23 (46%)	15 (30%)	8 (16%)
#PANCREATIC ACINUS	(49)	(47)	(49)
ATROPHY, NOS		2 (4%)	
#ESOPHAGUS	(49)	(50)	(47)
HYPERKERATOSIS		1 (2%)	1 (2%)
#STOMACH	(48)	(49)	(50)
HEMORRHAGE		1 (2%)	
INFLAMMATION, NOS			1 (2%)
ULCER, NOS		1 (2%)	
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	1 (2%)
NECROSIS, NOS		2 (4%)	1 (2%)
NECROSIS, FOCAL		3 (6%)	6 (12%)
HYPERPLASIA, EPITHELIAL		2 (4%)	7 (14%)
HYPERPLASIA, BASAL CELL		1 (2%)	1 (2%)
HYPERKERATOSIS	2 (4%)	6 (12%)	7 (14%)
ACANTHOSIS		1 (2%)	
#PEYER'S PATCH	(49)	(50)	(49)
HYPERPLASIA, NOS	9 (18%)	5 (10%)	5 (10%)
#COLON	(47)	(48)	(49)
PARASITISM	1 (2%)	2 (4%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
MINERALIZATION	7 (14%)	6 (12%)	4 (8%)
INFLAMMATION, NOS	7 (14%)	5 (10%)	2 (4%)
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
FIBROSIS, DIFFUSE	4 (8%)	2 (4%)	3 (6%)
NEPHROPATHY	42 (84%)	41 (82%)	36 (72%)
NECROSIS, FOCAL	1 (2%)	1 (2%)	
#RENAL PAPILLA	(50)	(50)	(50)
MINERALIZATION		1 (2%)	2 (4%)
NECROSIS, NOS	1 (2%)	1 (2%)	
#KIDNEY/TUBULE	(50)	(50)	(50)
NECROSIS, FOCAL			1 (2%)
#KIDNEY/PELVIS	(50)	(50)	(50)
HYPERPLASIA, EPITHELIAL	2 (4%)		
#URINARY BLADDER	(48)	(50)	(49)
HEMORRHAGE	1 (2%)		1 (2%)
INFLAMMATION, ACUTE	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
NECROSIS, NOS	1 (2%)	1 (2%)	

TABLE C2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(50)	(48)	(49)
DILATATION, NOS	4 (8%)	4 (8%)	7 (14%)
HEMORRHAGE	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, NOS	1 (2%)	7 (15%)	5 (10%)
HYPERPLASIA, FOCAL	3 (6%)		
#ADRENAL	(50)	(50)	(49)
DILATATION, NOS			3 (6%)
HEMORRHAGE	1 (2%)	1 (2%)	
INFLAMMATION, NOS			1 (2%)
DEGENERATION, NOS	1 (2%)		3 (6%)
NECROSIS, NOS		1 (2%)	1 (2%)
NECROSIS, FOCAL	2 (4%)		
INFARCT, NOS	1 (2%)		1 (2%)
METAMORPHOSIS FATTY	4 (8%)	7 (14%)	11 (22%)
#ADRENAL CORTEX	(50)	(50)	(49)
NECROSIS, NOS		1 (2%)	
INFARCT, NOS		1 (2%)	
METAMORPHOSIS FATTY			1 (2%)
HYPERTROPHY, FOCAL	8 (16%)	3 (6%)	5 (10%)
HYPERPLASIA, NOS	1 (2%)		1 (2%)
#ADRENAL MEDULLA	(50)	(50)	(49)
HYPERPLASIA, NOS	4 (8%)	6 (12%)	2 (4%)
HYPERPLASIA, FOCAL	2 (4%)	2 (4%)	1 (2%)
#THYROID	(47)	(48)	(50)
FOLLICULAR CYST, NOS		3 (6%)	2 (4%)
NECROSIS, NOS		1 (2%)	
HYPERPLASIA, C-CELL	7 (15%)	4 (8%)	
HYPERPLASIA, FOLLICULAR-CELL		2 (4%)	
#PANCREATIC ISLETS	(49)	(47)	(49)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
GALACTOCELE	11 (22%)	13 (26%)	12 (24%)
INFLAMMATION, NOS			3 (6%)
NECROSIS, NOS			3 (6%)
HYPERPLASIA, NOS	2 (4%)		
HYPERPLASIA, EPITHELIAL	1 (2%)		
*CLITORAL GLAND	(50)	(50)	(50)
INFLAMMATION, NOS	2 (4%)	2 (4%)	
NECROSIS, NOS	4 (8%)	3 (6%)	3 (6%)
HYPERKERATOSIS	1 (2%)		
ACANTHOSIS	1 (2%)		
#UTERUS	(50)	(50)	(49)
MINERALIZATION		1 (2%)	
HYDROMETRA	1 (2%)	6 (12%)	3 (6%)
HEMORRHAGE	2 (4%)	3 (6%)	1 (2%)
INFLAMMATION, NOS	1 (2%)	5 (10%)	2 (4%)
INFLAMMATION, ACUTE	1 (2%)	1 (2%)	
NECROSIS, NOS	1 (2%)	2 (4%)	1 (2%)
HYPERPLASIA, ADENOMATOUS		1 (2%)	1 (2%)
DECIDUAL ALTERATION, NOS	1 (2%)		
#UTERUS/ENDOMETRIUM	(50)	(50)	(49)
HYPERPLASIA, NOS		2 (4%)	1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		2 (4%)
HYPERPLASIA, CYSTIC		1 (2%)	

TABLE C2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(50)	(49)	(50)
HYDROCEPHALUS, NOS			1 (2%)
GLIOSIS			1 (2%)
SPECIAL SENSE ORGANS			
*EYE ANTERIOR CHAMBER	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
*EYE/CORNEA	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
*EAR	(50)	(50)	(50)
NECROSIS, NOS			1 (2%)
HYPERKERATOSIS			1 (2%)
*ZYMBAL GLAND	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
INFLAMMATION, NOS			3 (6%)
NECROSIS, NOS		1 (2%)	5 (10%)
HYPERKERATOSIS		2 (4%)	3 (6%)
ACANTHOSIS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
NECROSIS, NOS			1 (2%)
*PERICARDIUM	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
MINERALIZATION			1 (2%)
INFLAMMATION, NOS		2 (4%)	
NECROSIS, NOS		1 (2%)	1 (2%)
HYPERKERATOSIS			1 (2%)
OMENTUM			
STEATITIS	1		
NECROSIS, FAT	2		1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
AUTO/NECROPSY/HISTO PERFORMED			1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
INFLAMMATION, NECROTIZING			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
ABSCESS, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
CONGESTION, NOS	1 (2%)		1 (2%)
HEMORRHAGE	1 (2%)	5 (10%)	3 (6%)
INFLAMMATION, NOS	3 (6%)		2 (4%)
INFLAMMATION, MULTIFOCAL	1 (2%)		
INFLAMMATION, ACUTE	1 (2%)	2 (4%)	1 (2%)
INFLAMMATION, ACUTE DIFFUSE	1 (2%)		
INFLAMMATION, CHRONIC			1 (2%)
ALVEOLAR MACROPHAGES	7 (14%)	4 (8%)	3 (6%)
HYPERPLASIA, EPITHELIAL	2 (4%)	2 (4%)	4 (8%)
HISTIOCYTOSIS	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MASTOCYTOSIS		1 (2%)	
HEMATOPOIESIS	2 (4%)		5 (10%)
MYELOID METAPLASIA	1 (2%)		
#BONE MARROW	(49)	(47)	(47)
OSTEOSCLEROSIS		1 (2%)	
HEMATOPOIESIS	1 (2%)	2 (4%)	1 (2%)
#SPLEEN	(49)	(50)	(50)
MINERALIZATION		1 (2%)	
FIBROSIS	2 (4%)		
ANGIECTASIS	1 (2%)		
HYPERPLASIA, LYMPHOID	3 (6%)	1 (2%)	2 (4%)
HEMATOPOIESIS	16 (33%)	28 (56%)	24 (48%)
#LYMPH NODE	(48)	(45)	(47)
INFLAMMATION, ACUTE			1 (2%)
GRANULOMA, NOS			1 (2%)
NECROSIS, NOS	1 (2%)		1 (2%)
NECROSIS, FOCAL			1 (2%)
ANGIECTASIS	7 (15%)	6 (13%)	10 (21%)
PLASMACYTOSIS	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, LYMPHOID	5 (10%)	2 (4%)	
MASTOCYTOSIS			1 (2%)
HEMATOPOIESIS	9 (19%)	4 (9%)	11 (23%)
#PANCREATIC LYMPH NODE	(48)	(45)	(47)
MINERALIZATION	1 (2%)		
FIBROSIS	1 (2%)		
#MESENTERIC LYMPH NODE	(48)	(45)	(47)
FIBROSIS	1 (2%)		
#LIVER	(50)	(50)	(50)
HEMATOPOIESIS	8 (16%)	2 (4%)	3 (6%)

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#STOMACH	(50)	(50)	(50)
MASTOCYTOSIS			1 (2%)
#PEYER'S PATCH	(50)	(48)	(47)
HYPERPLASIA, LYMPHOID	5 (10%)	2 (4%)	1 (2%)
#RENAL PAPILLA	(50)	(50)	(50)
MASTOCYTOSIS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(50)
MINERALIZATION		1 (2%)	1 (2%)
INFLAMMATION, NOS			2 (4%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
#MYOCARDIUM	(50)	(50)	(50)
DEGENERATION, NOS	3 (6%)	2 (4%)	
#ENDOCARDIUM	(50)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
*PANCREATIC ARTERY	(50)	(50)	(50)
PERIVASCULITIS		1 (2%)	
#LIVER	(50)	(50)	(50)
THROMBOSIS, NOS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(50)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
#LIVER	(50)	(50)	(50)
MINERALIZATION	2 (4%)	6 (12%)	9 (18%)
HEMORRHAGE	1 (2%)	2 (4%)	
INFLAMMATION, ACUTE		1 (2%)	
FIBROSIS	1 (2%)	2 (4%)	
CHOLANGIOFIBROSIS			1 (2%)
DEGENERATION, NOS			1 (2%)
NECROSIS, NOS	5 (10%)	11 (22%)	11 (22%)
NECROSIS, FOCAL	19 (38%)	5 (10%)	7 (14%)
NECROSIS, ISCHEMIC		3 (6%)	3 (6%)
INFARCT, NOS	3 (6%)	8 (16%)	13 (26%)
METAMORPHOSIS FATTY	19 (38%)	16 (32%)	12 (24%)
CYTOPLASMIC CHANGE, NOS	5 (10%)	6 (12%)	
FOCAL CELLULAR CHANGE		1 (2%)	
EOSINOPHILIC CYTO CHANGE	1 (2%)		1 (2%)
CLEAR-CELL CHANGE		1 (2%)	
*GALLBLADDER	(50)	(50)	(50)
INFLAMMATION, NOS	1 (2%)		
#PANCREATIC ACINUS	(50)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)	
#STOMACH	(50)	(50)	(50)
MINERALIZATION	1 (2%)	1 (2%)	
INFLAMMATION, NOS	1 (2%)	4 (8%)	2 (4%)
INFLAMMATION, ACUTE			1 (2%)
NECROSIS, NOS		1 (2%)	1 (2%)
NECROSIS, FOCAL		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, EPITHELIAL	1 (2%)		
HYPERKERATOSIS	1 (2%)	6 (12%)	4 (8%)
#GASTRIC MUCOSA	(50)	(50)	(50)
HYPERPLASIA, NOS	2 (4%)		
#GASTRIC SUBMUCOSA	(50)	(50)	(50)
FIBROSIS			1 (2%)

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM (Continued)			
#SMALL INTESTINE	(50)	(48)	(47)
NECROSIS, NOS			1 (2%)
#JEJUNUM	(50)	(48)	(47)
NECROSIS, NOS	1 (2%)		
#COLONIC MUCOSA	(49)	(47)	(47)
INFLAMMATION, NECROTIZING	1 (2%)		
#CECUM	(49)	(47)	(47)
NECROSIS, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
MINERALIZATION	34 (68%)	32 (64%)	24 (48%)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, ACUTE		1 (2%)	1 (2%)
FIBROSIS	2 (4%)		
NEPHROPATHY	15 (30%)	11 (22%)	7 (14%)
CYTOPLASMIC VACUOLIZATION			3 (6%)
#RENAL PAPILLA	(50)	(50)	(50)
NECROSIS, NOS			2 (4%)
#KIDNEY/TUBULE	(50)	(50)	(50)
INFLAMMATION, ACUTE	1 (2%)		
FIBROSIS	1 (2%)		
DEGENERATION, NOS	1 (2%)	1 (2%)	
NECROSIS, FOCAL	1 (2%)		
#URINARY BLADDER	(50)	(50)	(50)
CALCULUS, MICROSCOPIC EXAM	1 (2%)		4 (8%)
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(47)	(48)
HYPERPLASIA, NOS	3 (7%)	1 (2%)	2 (4%)
ANGIECTASIS			1 (2%)
#ADRENAL	(50)	(50)	(50)
NECROSIS, NOS		1 (2%)	
ANGIECTASIS			1 (2%)
#ADRENAL/CAPSULE	(50)	(50)	(50)
HYPERPLASIA, NOS	17 (34%)	15 (30%)	8 (16%)
HYPERPLASIA, FOCAL	1 (2%)		
#ADRENAL CORTEX	(50)	(50)	(50)
HYPERTROPHY, NOS	3 (6%)	3 (6%)	
HYPERTROPHY, FOCAL	3 (6%)	1 (2%)	4 (8%)
HYPERPLASIA, NOS	2 (4%)	3 (6%)	
#ADRENAL MEDULLA	(50)	(50)	(50)
HYPERPLASIA, NOS		2 (4%)	3 (6%)
#THYROID	(50)	(48)	(49)
FOLLICULAR CYST, NOS		2 (4%)	
INFLAMMATION, NOS	1 (2%)		
HYPERPLASIA, FOLLICULAR-CELL	2 (4%)	2 (4%)	3 (6%)
#PANCREATIC ISLETS	(50)	(50)	(49)
HYPERPLASIA, NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(50)
INFLAMMATION, NOS	1 (2%)	2 (4%)	4 (8%)
NECROSIS, NOS			2 (4%)
HYPERPLASIA, NOS			1 (2%)
HYPERKERATOSIS		1 (2%)	2 (4%)

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM (Continued)			
#PROSTATE	(46)	(45)	(46)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, NECROTIZING			1 (2%)
*SEMINAL VESICLE	(50)	(50)	(50)
INFLAMMATION, NECROTIZING	1 (2%)		
#TESTIS	(49)	(48)	(49)
MINERALIZATION	2 (4%)	1 (2%)	
SPERMATOCELE		1 (2%)	
INFLAMMATION, NOS	1 (2%)		
MULTINUCLEATE GIANT-CELL		1 (2%)	1 (2%)
ATROPHY, NOS	3 (6%)	2 (4%)	1 (2%)
#TESTIS/TUBULE	(49)	(48)	(49)
MINERALIZATION		1 (2%)	
SPERMATOCELE		1 (2%)	
DEGENERATION, NOS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(50)	(49)	(50)
HEMORRHAGE		3 (6%)	1 (2%)
INFLAMMATION, NOS	1 (2%)		
MALACIA	1 (2%)		
SPECIAL SENSE ORGANS			
*ZYMBAL GLAND	(50)	(50)	(50)
INFLAMMATION, NOS	1 (2%)		
NECROSIS, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*MANDIBLE	(50)	(50)	(50)
NECROSIS, NOS			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
ABSCESS, NOS			1 (2%)
FIBROSIS			1 (2%)
NECROSIS, NOS			2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
CONGESTION, NOS		3 (6%)	2 (4%)
INFLAMMATION, NOS	2 (4%)		
AMYLOIDOSIS	1 (2%)		
OMENTUM			
MINERALIZATION	1		
NECROSIS, FAT	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		2	

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

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
NECROSIS, NOS		1 (2%)	
HYPERKERATOSIS		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(50)
HEMORRHAGE	1 (2%)		
INFLAMMATION, NOS	1 (2%)		
NECROSIS, NOS	1 (2%)		
RESPIRATORY SYSTEM			
*NASAL CAVITY	(50)	(50)	(50)
INFLAMMATION, ACUTE FOCAL			1 (2%)
#LUNG	(50)	(49)	(47)
MINERALIZATION			2 (4%)
CONGESTION, NOS	2 (4%)		
HEMORRHAGE	1 (2%)	1 (2%)	4 (9%)
INFLAMMATION, NOS	4 (8%)	1 (2%)	1 (2%)
INFLAMMATION, FOCAL	1 (2%)		
INFLAMMATION, NECROTIZING	1 (2%)		
INFLAMMATION, ACUTE	3 (6%)	2 (4%)	1 (2%)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		1 (2%)
FIBROSIS			1 (2%)
HYPERPLASIA, EPITHELIAL	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MASTOCYTOSIS		1 (2%)	
HEMATOPOIESIS	4 (8%)		2 (4%)
MYELOID METAPLASIA		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(50)
MASTOCYTOSIS			1 (2%)
#BONE MARROW	(38)	(44)	(41)
MYELOFIBROSIS		1 (2%)	
HEMATOPOIESIS	4 (11%)	2 (5%)	
#SPLEEN	(49)	(47)	(46)
MINERALIZATION	1 (2%)		1 (2%)
DILATATION, NOS			1 (2%)
HEMORRHAGE	1 (2%)		
FIBROSIS, FOCAL	1 (2%)		
NECROSIS, NOS	2 (4%)	1 (2%)	
LYMPHOID DEPLETION	2 (4%)		2 (4%)
HYPERPLASIA, LYMPHOID	10 (20%)	3 (6%)	
MASTOCYTOSIS		1 (2%)	
HEMATOPOIESIS	27 (55%)	22 (47%)	29 (63%)
#SPLENIC FOLLICLES	(49)	(47)	(46)
ATROPHY, NOS			1 (2%)
#LYMPH NODE	(46)	(46)	(40)
HEMORRHAGE			1 (3%)
INFLAMMATION, NOS	3 (7%)		
NECROSIS, NOS			1 (3%)
LYMPHOID DEPLETION			1 (3%)
ANGIECTASIS	5 (11%)	6 (13%)	4 (10%)

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM			
#LYMPH NODE (Continued)	(46)	(46)	(40)
PLASMACYTOSIS	3 (7%)	1 (2%)	
HYPERPLASIA, RETICULUM CELL			1 (3%)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	1 (3%)
HEMATOPOIESIS	4 (9%)	4 (9%)	5 (13%)
*COSTOCHONDRAL SYNCHONDROSIS	(50)	(50)	(50)
MASTOCYTOSIS		1 (2%)	
#LIVER	(49)	(50)	(49)
HEMATOPOIESIS	4 (8%)	1 (2%)	1 (2%)
MYELOID METAPLASIA	1 (2%)		
#GASTRIC MUSCULARIS	(48)	(46)	(46)
MASTOCYTOSIS		1 (2%)	
#KIDNEY	(50)	(50)	(48)
MASTOCYTOSIS		1 (2%)	
HEMATOPOIESIS		1 (2%)	
#ADRENAL	(48)	(47)	(45)
HEMATOPOIESIS	1 (2%)		
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
PERIVASCULITIS			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
PERIVASCULITIS	1 (2%)		
#LYMPH NODE	(46)	(46)	(40)
THROMBOSIS, NOS		1 (2%)	
#HEART	(50)	(49)	(47)
MINERALIZATION	1 (2%)	1 (2%)	4 (9%)
ENDOCARDITIS, BACTERIAL	1 (2%)		
INFLAMMATION, ACUTE			1 (2%)
NECROSIS, NOS			1 (2%)
#MYOCARDIUM	(50)	(49)	(47)
DEGENERATION, NOS	2 (4%)		1 (2%)
#HEPATIC SINUSOID	(49)	(50)	(49)
DILATATION, NOS			1 (2%)
#KIDNEY	(50)	(50)	(48)
THROMBOSIS, NOS	1 (2%)		
PERIVASCULITIS	1 (2%)		
#URINARY BLADDER	(48)	(47)	(45)
PERIVASCULITIS	1 (2%)		
#UTERUS	(50)	(50)	(43)
THROMBOSIS, NOS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(48)	(42)	(45)
INFLAMMATION, NOS			1 (2%)
#LIVER	(49)	(50)	(49)
MINERALIZATION		2 (4%)	5 (10%)
HEMORRHAGE			1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
FIBROSIS			1 (2%)
NECROSIS, NOS		7 (14%)	11 (22%)
NECROSIS, FOCAL	9 (18%)	2 (4%)	3 (6%)
NECROSIS, ISCHEMIC	1 (2%)	6 (12%)	12 (24%)
NECROSIS, HEMORRHAGIC			1 (2%)
METAMORPHOSIS FATTY	18 (37%)	12 (24%)	13 (27%)

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER (Continued)	(49)	(50)	(49)
CYTOPLASMIC VACUOLIZATION			1 (2%)
BASOPHILIC CYTO CHANGE		1 (2%)	
FOCAL CELLULAR CHANGE			1 (2%)
EOSINOPHILIC CYTO CHANGE		1 (2%)	
#BILE DUCT	(49)	(50)	(49)
HYPERPLASIA, NOS			1 (2%)
#PANCREATIC ACINUS	(46)	(45)	(38)
ATROPHY, NOS	1 (2%)	1 (2%)	
#STOMACH	(48)	(46)	(46)
MINERALIZATION	1 (2%)		
INFLAMMATION, NOS	4 (8%)	1 (2%)	3 (7%)
ULCER, NOS	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	3 (7%)
NECROSIS, NOS		1 (2%)	1 (2%)
NECROSIS, FOCAL	2 (4%)	2 (4%)	1 (2%)
HYPERPLASIA, EPITHELIAL			5 (11%)
HYPERKERATOSIS	10 (21%)	19 (41%)	16 (35%)
ACANTHOSIS	2 (4%)	3 (7%)	
#PEYER'S PATCH	(46)	(38)	(43)
HYPERPLASIA, NOS	5 (11%)		2 (5%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(48)
MINERALIZATION	1 (2%)	2 (4%)	4 (8%)
GLOMERULONEPHRITIS, NOS		1 (2%)	
INFLAMMATION, NOS	1 (2%)	1 (2%)	3 (6%)
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, NECROTIZING	1 (2%)		
INFLAMMATION, ACUTE	1 (2%)		
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
FIBROSIS	1 (2%)		
NEPHROPATHY	5 (10%)	8 (16%)	8 (17%)
GLOMERULOSCLEROSIS, NOS	2 (4%)	1 (2%)	1 (2%)
NECROSIS, NOS			1 (2%)
#RENAL PAPILLA	(50)	(50)	(48)
MINERALIZATION	2 (4%)		
NECROSIS, NOS	1 (2%)		
#URINARY BLADDER	(48)	(47)	(45)
INFLAMMATION, ACUTE	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(38)	(36)	(29)
DILATATION, NOS	3 (8%)		1 (3%)
HEMORRHAGE	2 (5%)	1 (3%)	
HYPERPLASIA, NOS	4 (11%)	3 (8%)	
HYPERPLASIA, FOCAL	1 (3%)		
#ADRENAL	(48)	(47)	(45)
METAMORPHOSIS FATTY		2 (4%)	
ANGIECTASIS		1 (2%)	
#ADRENAL/CAPSULE	(48)	(47)	(45)
HYPERPLASIA, NOS	26 (54%)	19 (40%)	9 (20%)
#ADRENAL CORTEX	(48)	(47)	(45)
HYPERTROPHY, NOS	1 (2%)		
HYPERPLASIA, NOS		1 (2%)	
#ADRENAL MEDULLA	(48)	(47)	(45)
HYPERPLASIA, NOS	1 (2%)		2 (4%)

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM (Continued)			
#THYROID	(45)	(41)	(35)
DEGENERATION, NOS	1 (2%)		
HYPERPLASIA, FOLLICULAR-CELL	2 (4%)	1 (2%)	
#PANCREATIC ISLETS	(46)	(45)	(38)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(50)	(50)	(43)
HYDROMETRA	13 (26%)	8 (16%)	3 (7%)
HEMORRHAGE		1 (2%)	
HEMATOMETRA		1 (2%)	
INFLAMMATION, NOS	6 (12%)		2 (5%)
INFLAMMATION, NECROTIZING	1 (2%)		
ABSCESS, NOS		1 (2%)	
NECROSIS, NOS			1 (2%)
ANGIECTASIS	1 (2%)	3 (6%)	1 (2%)
#UTERUS/ENDOMETRIUM	(50)	(50)	(43)
HYPERPLASIA, NOS	2 (4%)	1 (2%)	1 (2%)
HYPERPLASIA, CYSTIC	26 (52%)	6 (12%)	1 (2%)
#OVARY	(48)	(46)	(41)
MINERALIZATION		5 (11%)	1 (2%)
HEMORRHAGE		3 (7%)	2 (5%)
ABSCESS, NOS	7 (15%)	2 (4%)	1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
REACTION, FOREIGN BODY		1 (2%)	
ANGIECTASIS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*THORACIC CAVITY	(50)	(50)	(50)
INFLAMMATION, NECROTIZING	1 (2%)	1 (2%)	
*PERITONEUM	(50)	(50)	(50)
INFLAMMATION, NOS	2 (4%)	1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
INFLAMMATION, NOS	1 (2%)	1 (2%)	
OMENTUM			
MINERALIZATION	1		
NECROSIS, FAT	6	1	1
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERFORMED			1

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

APPENDIX E

ANALYSES OF PRIMARY TUMORS IN RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF C. I. BASIC RED 9 MONOHYDROCHLORIDE

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	1,000 ppm	2,000 ppm
Skin: Basal Cell Carcinoma			
Overall Rates (a)	1/50 (2%)	0/50 (0%)	4/50 (6%)
Adjusted Rates (b)	2.8%	0.0%	43.4%
Terminal Rates (c)	1/36 (3%)	0/29 (0%)	0/0
Life Table Tests (d)	P<0.001	P=0.543N	P<0.001
Incidental Tumor Tests (d)	P=0.100	P=0.543N	P=0.206
Cochran-Armitage Trend Test (d)	P=0.082		
Fisher Exact Tests		P=0.500N	P=0.181
Skin: Squamous Cell Papilloma			
Overall Rates (a)	2/50 (4%)	1/50 (2%)	4/50 (8%)
Adjusted Rates (b)	5.6%	3.4%	28.8%
Terminal Rates (c)	2/36 (6%)	1/29 (3%)	0/0
Life Table Tests (d)	P=0.007	P=0.576N	P=0.005
Incidental Tumor Tests (d)	P=0.176	P=0.576N	P=0.288
Cochran-Armitage Trend Test (d)	P=0.238		
Fisher Exact Tests		P=0.500N	P=0.339
Skin: Squamous Cell Carcinoma			
Overall Rates (a)	0/50 (0%)	1/50 (2%)	10/50 (20%)
Adjusted Rates (b)	0.0%	3.4%	85.4%
Terminal Rates (c)	0/36 (0%)	1/29 (3%)	0/0
Life Table Tests (d)	P<0.001	P=0.457	P<0.001
Incidental Tumor Tests (d)	P<0.001	P=0.457	P=0.019
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.500	P<0.001
Skin: Squamous Cell Papilloma or Carcinoma			
Overall Rates (a)	2/50 (4%)	2/50 (4%)	14/50 (28%)
Adjusted Rates (b)	5.6%	6.9%	89.6%
Terminal Rates (c)	2/36 (6%)	2/29 (7%)	0/0
Life Table Tests (d)	P<0.001	P=0.615	P<0.001
Incidental Tumor Tests (d)	P<0.001	P=0.615	P=0.005
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.691	P<0.001
Skin: Trichoepithelioma			
Overall Rates (a)	0/50 (0%)	0/50 (0%)	7/50 (14%)
Adjusted Rates (b)	0.0%	0.0%	71.1%
Terminal Rates (c)	0/36 (0%)	0/29 (0%)	0/0
Life Table Tests (d)	P<0.001	(e)	P<0.001
Incidental Tumor Tests (d)	P=0.006	(e)	P=0.061
Cochran-Armitage Trend Test (d)	P=0.001		
Fisher Exact Tests		(e)	P=0.006
Skin: Sebaceous Adenoma			
Overall Rates (a)	0/50 (0%)	0/50 (0%)	5/50 (10%)
Adjusted Rates (b)	0.0%	0.0%	27.3%
Terminal Rates (c)	0/36 (0%)	0/29 (0%)	0/0
Life Table Tests (d)	P<0.001	(e)	P=0.001
Incidental Tumor Tests (d)	P=0.057	(e)	P=0.224
Cochran-Armitage Trend Test (d)	P=0.006		
Fisher Exact Tests		(e)	P=0.028
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	2/50 (4%)	20/50 (40%)	16/50 (32%)
Adjusted Rates (b)	5.6%	47.8%	100.0%
Terminal Rates (c)	2/36 (6%)	9/29 (31%)	0/0
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P=0.001	P<0.001	P=0.004
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	1,000 ppm	2,000 ppm
Subcutaneous Tissue: Sarcoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	4/50 (8%)
Adjusted Rates (b)	6.7%	5.2%	56.8%
Terminal Rates (c)	1/36 (3%)	0/29 (0%)	0/0
Life Table Tests (d)	P=0.060	P=0.537N	P=0.066
Incidental Tumor Tests (d)	P=0.288N	P=0.491N	P=0.419N
Cochran-Armitage Trend Test (d)	P=0.417		
Fisher Exact Tests		P=0.500N	P=0.500
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	2.4%	10.3%	0.0%
Terminal Rates (c)	0/36 (0%)	3/29 (10%)	0/0
Life Table Tests (d)	P=0.304	P=0.243	P=0.823N
Incidental Tumor Tests (d)	P=0.492	P=0.287	P=0.410N
Cochran-Armitage Trend Test (d)	P=0.378N		
Fisher Exact Tests		P=0.309	P=0.500N
Subcutaneous: Sarcoma or Fibrosarcoma			
Overall Rates (a)	4/50 (8%)	5/50 (10%)	4/50 (8%)
Adjusted Rates (b)	8.9%	15.0%	56.8%
Terminal Rates (c)	1/36 (3%)	3/29 (10%)	0/0
Life Table Tests (d)	P=0.033	P=0.422	P=0.099
Incidental Tumor Tests (d)	P=0.394N	P=0.492	P=0.223N
Cochran-Armitage Trend Test (d)	P=0.571		
Fisher Exact Tests		P=0.500	P=0.643
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	3/50 (6%)	22/50 (44%)	16/50 (32%)
Adjusted Rates (b)	7.8%	53.0%	100.0%
Terminal Rates (c)	2/36 (6%)	11/29 (38%)	0/0
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P=0.001	P<0.001	P=0.014
Cochran-Armitage Trend Test (d)	P=0.003		
Fisher Exact Tests		P<0.001	P<0.001
Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates (a)	6/50 (12%)	24/50 (48%)	19/50 (38%)
Adjusted Rates (b)	14.1%	55.5%	100.0%
Terminal Rates (c)	3/36 (8%)	11/29 (38%)	0/0
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P=0.013	P<0.001	P=0.086
Cochran-Armitage Trend Test (d)	P=0.004		
Fisher Exact Tests		P<0.001	P=0.002
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	0.0%	8.9%	8.4%
Terminal Rates (c)	0/36 (0%)	2/29 (7%)	0/0
Life Table Tests (d)	P=0.008	P=0.101	P=0.076
Incidental Tumor Tests (d)	P=0.223	P=0.132	P=0.590
Cochran-Armitage Trend Test (d)	P=0.101		
Fisher Exact Tests		P=0.121	P=0.121
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	4/50 (8%)
Adjusted Rates (b)	2.4%	8.9%	31.3%
Terminal Rates (c)	0/36 (0%)	2/29 (7%)	0/0
Life Table Tests (d)	P=0.004	P=0.258	P=0.017
Incidental Tumor Tests (d)	P=0.319	P=0.367	P=0.656
Cochran-Armitage Trend Test (d)	P=0.133		
Fisher Exact Tests		P=0.309	P=0.181

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	1,000 ppm	2,000 ppm
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	5/50 (10%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	13.1%	3.4%	5.6%
Terminal Rates (c)	4/36 (11%)	1/29 (3%)	0/0
Life Table Tests (d)	P=0.459N	P=0.153N	P=0.498
Incidental Tumor Tests (d)	P=0.156N	P=0.117N	P=0.461N
Cochran-Armitage Trend Test (d)	P=0.049N		
Fisher Exact Tests		P=0.102N	P=0.102N
Hematopoietic System: Leukemia			
Overall Rates (a)	7/50 (14%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	17.0%	3.4%	5.6%
Terminal Rates (c)	4/36 (11%)	1/29 (3%)	0/0
Life Table Tests (d)	P=0.187N	P=0.056N	P=0.719
Incidental Tumor Tests (d)	P=0.013N	P=0.023N	P=0.042N
Cochran-Armitage Trend Test (d)	P=0.010N		
Fisher Exact Tests		P=0.030N	P=0.030N
Liver: Bile Duct Adenoma or Carcinoma			
Overall Rates (a)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted Rates (b)	0.0%	0.0%	25.5%
Terminal Rates (c)	0/36 (0%)	0/29 (0%)	0/0
Life Table Tests (d)	P=0.002	(e)	P=0.005
Incidental Tumor Tests (d)	P=0.079	(e)	P=0.263
Cochran-Armitage Trend Test (d)	P=0.037		
Fisher Exact Tests		(e)	P=0.121
Liver: Neoplastic Nodule			
Overall Rates (a)	5/50 (10%)	14/50 (28%)	6/50 (12%)
Adjusted Rates (b)	13.9%	46.3%	38.9%
Terminal Rates (c)	5/36 (14%)	13/29 (45%)	0/0
Life Table Tests (d)	P<0.001	P=0.004	P<0.001
Incidental Tumor Tests (d)	P=0.002	P=0.005	P=0.198
Cochran-Armitage Trend Test (d)	P=0.447		
Fisher Exact Tests		P=0.020	P=0.500
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	0/50 (0%)	2/50 (4%)	8/50 (16%)
Adjusted Rates (b)	0.0%	6.9%	57.4%
Terminal Rates (c)	0/36 (0%)	2/29 (7%)	0/0
Life Table Tests (d)	P<0.001	P=0.192	P<0.001
Incidental Tumor Tests (d)	P<0.001	P=0.192	P=0.035
Cochran-Armitage Trend Test (d)	P=0.001		
Fisher Exact Tests		P=0.247	P=0.003
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall Rates (a)	5/50 (10%)	15/50 (30%)	14/50 (28%)
Adjusted Rates (b)	13.9%	49.6%	74.0%
Terminal Rates (c)	5/36 (14%)	14/29 (48%)	0/0
Life Table Tests (d)	P<0.001	P=0.002	P<0.001
Incidental Tumor Tests (d)	P<0.001	P=0.002	P=0.007
Cochran-Armitage Trend Test (d)	P=0.021		
Fisher Exact Tests		P=0.011	P=0.020
Pituitary: Adenoma			
Overall Rates (a)	17/48 (35%)	16/47 (34%)	8/46 (17%)
Adjusted Rates (b)	42.8%	43.1%	54.3%
Terminal Rates (c)	13/35 (37%)	8/27 (30%)	0/0 (0%)
Life Table Tests (d)	P=0.006	P=0.408	P<0.001
Incidental Tumor Tests (d)	P=0.222N	P=0.438N	P=0.454N
Cochran-Armitage Trend Test (d)	P=0.036N		
Fisher Exact Tests		P=0.530N	P=0.040N

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	1,000 ppm	2,000 ppm
Pituitary: Adenoma or Carcinoma			
Overall Rates (a)	17/48 (35%)	17/47 (36%)	8/46 (17%)
Adjusted Rates (b)	42.8%	44.9%	54.3%
Terminal Rates (c)	13/35 (37%)	8/27 (30%)	0/0 (0%)
Life Table Tests (d)	P=0.005	P=0.331	P<0.001
Incidental Tumor Tests (d)	P=0.210N	P=0.499N	P=0.454N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Tests		P=0.554	P=0.040N
Adrenal: Pheochromocytoma			
Overall Rates (a)	10/49 (20%)	14/49 (29%)	3/48 (6%)
Adjusted Rates (b)	26.5%	44.5%	16.5%
Terminal Rates (c)	9/36 (25%)	12/29 (41%)	0/0
Life Table Tests (d)	P=0.015	P=0.098	P=0.120
Incidental Tumor Tests (d)	P=0.235	P=0.118	P=0.671N
Cochran-Armitage Trend Test (d)	P=0.049N		
Fisher Exact Tests		P=0.241	P=0.039N
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant			
Overall Rates (a)	11/49 (22%)	14/49 (29%)	3/48 (6%)
Adjusted Rates (b)	29.2%	44.5%	16.5%
Terminal Rates (c)	10/36 (28%)	12/29 (41%)	0/0
Life Table Tests (d)	P=0.024	P=0.143	P=0.120
Incidental Tumor Tests (d)	P=0.292	P=0.169	P=0.671N
Cochran-Armitage Trend Test (d)	P=0.030N		
Fisher Exact Tests		P=0.322	P=0.022N
Thyroid: Follicular Cell Adenoma			
Overall Rates (a)	0/49 (0%)	0/46 (0%)	9/44 (20%)
Adjusted Rates (b)	0.0%	0.0%	78.4%
Terminal Rates (c)	0/36 (0%)	0/27 (0%)	0/0
Life Table Tests (d)	P<0.001	(e)	P<0.001
Incidental Tumor Tests (d)	P=0.004	(e)	P=0.038
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		(e)	P<0.001
Thyroid: Follicular Cell Carcinoma			
Overall Rates (a)	0/49 (0%)	5/46 (11%)	18/44 (41%)
Adjusted Rates (b)	0.0%	15.9%	81.6%
Terminal Rates (c)	0/36 (0%)	3/27 (11%)	0/0
Life Table Tests (d)	P<0.001	P=0.020	P<0.001
Incidental Tumor Tests (d)	P<0.001	P=0.030	P=0.010
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.024	P<0.001
Thyroid: Follicular Cell Adenoma or Carcinoma			
Overall Rates (a)	0/49 (0%)	5/46 (11%)	25/44 (57%)
Adjusted Rates (b)	0.0%	15.9%	91.4%
Terminal Rates (c)	0/36 (0%)	3/27 (11%)	0/0
Life Table Tests (d)	P<0.001	P=0.020	P<0.001
Incidental Tumor Tests (d)	P<0.001	P=0.030	P=0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.024	P<0.001
Thyroid: C-Cell Adenoma			
Overall Rates (a)	4/49 (8%)	2/46 (4%)	0/44 (0%)
Adjusted Rates (b)	11.1%	6.5%	0.0%
Terminal Rates (c)	4/36 (11%)	1/27 (4%)	0/0
Life Table Tests (d)	P=0.451N	P=0.463N	(f)
Incidental Tumor Tests (d)	P=0.277N	P=0.422N	(f)
Cochran-Armitage Trend Test (d)	P=0.047N		
Fisher Exact Tests		P=0.369N	P=0.073N

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	1,000 ppm	2,000 ppm
Thyroid: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	4/49 (8%)	3/46 (7%)	1/44 (2%)
Adjusted Rates (b)	11.1%	10.1%	14.3%
Terminal Rates (c)	4/36 (11%)	2/27 (7%)	0/0
Life Table Tests (d)	P=0.280	P=0.643N	P=0.158
Incidental Tumor Tests (d)	P=0.588	P=0.606N	P=0.590
Cochran-Armitage Trend Test (d)	P=0.162N		
Fisher Exact Tests		P=0.536N	P=0.216N
Pancreatic Islets: Islet Cell Adenoma			
Overall Rates (a)	2/47 (4%)	2/50 (4%)	3/46 (7%)
Adjusted Rates (b)	5.1%	5.6%	32.5%
Terminal Rates (c)	1/36 (3%)	1/29 (3%)	0/0
Life Table Tests (d)	P=0.014	P=0.634	P=0.003
Incidental Tumor Tests (d)	P=0.390	P=0.604N	P=0.579
Cochran-Armitage Trend Test (d)	P=0.396		
Fisher Exact Tests		P=0.668N	P=0.490
Pancreatic Islets: Islet Cell Carcinoma			
Overall Rates (a)	0/47 (0%)	3/50 (6%)	1/46 (2%)
Adjusted Rates (b)	0.0%	10.3%	10.0%
Terminal Rates (c)	0/36 (0%)	3/29 (10%)	0/0
Life Table Tests (d)	P=0.010	P=0.085	P=0.217
Incidental Tumor Tests (d)	P=0.040	P=0.085	P=0.638
Cochran-Armitage Trend Test (d)	P=0.370		
Fisher Exact Tests		P=0.133	P=0.495
Pancreatic Islets: Islet Cell Adenoma or Carcinoma			
Overall Rates (a)	2/47 (4%)	5/50 (10%)	4/46 (9%)
Adjusted Rates (b)	5.1%	5.8%	40.0%
Terminal Rates (c)	1/36 (3%)	4/29 (14%)	0/0
Life Table Tests (d)	P<0.001	P=0.152	P<0.001
Incidental Tumor Tests (d)	P=0.070	P=0.234	P=0.426
Cochran-Armitage Trend Test (d)	P=0.270		
Fisher Exact Tests		P=0.244	P=0.328
Mammary Gland: Fibroadenoma			
Overall Rates (a)	4/50 (8%)	6/50 (12%)	2/50 (4%)
Adjusted Rates (b)	10.0%	19.3%	53.3%
Terminal Rates (c)	1/36 (3%)	5/29 (17%)	0/0
Life Table Tests (d)	P=0.043	P=0.271	P=0.101
Incidental Tumor Tests (d)	P=0.547	P=0.398	P=0.230N
Cochran-Armitage Trend Test (d)	P=0.290N		
Fisher Exact Tests		P=0.370	P=0.339N
Mammary Gland: Adenoma or Fibroadenoma			
Overall Rates (a)	5/50 (10%)	5/50 (12%)	2/50 (4%)
Adjusted Rates (b)	12.5%	19.3%	53.3%
Terminal Rates (c)	2/36 (6%)	5/29 (17%)	0/0
Life Table Tests (d)	P=0.075	P=0.379	P=0.101
Incidental Tumor Tests (d)	P=0.556N	P=0.518	P=0.230N
Cochran-Armitage Trend Test (d)	P=0.187N		
Fisher Exact Tests		P=0.500	P=0.218N
Preputial Gland: Adenoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	8.3%	4.5%	4.2%
Terminal Rates (c)	3/36 (8%)	0/29 (0%)	0/0
Life Table Tests (d)	P=0.514	P=0.562N	P=0.366
Incidental Tumor Tests (d)	P=0.253N	P=0.436N	P=0.748
Cochran-Armitage Trend Test (d)	P=0.222N		
Fisher Exact Tests		P=0.500N	P=0.309N

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	1,000 ppm	2,000 ppm
Preputial Gland: Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted Rates (b)	11.1%	4.5%	9.5%
Terminal Rates (c)	4/36 (11%)	0/29 (0%)	0/0
Life Table Tests (d)	P=0.332	P=0.410N	P=0.090
Incidental Tumor Tests (d)	P=0.313N	P=0.297N	P=0.539
Cochran-Armitage Trend Test (d)	P=0.252N		
Fisher Exact Tests		P=0.339N	P=0.339N
Testis: Interstitial Cell Tumor			
Overall Rates (a)	43/48 (90%)	46/48 (96%)	37/50 (74%)
Adjusted Rates (b)	97.7%	100.0%	100.0%
Terminal Rates (c)	35/36 (97%)	29/29 (100%)	0/0
Life Table Tests (d)	P<0.001	P=0.026	P<0.001
Incidental Tumor Tests (d)	P=0.033	P=0.169	P=0.089
Cochran-Armitage Trend Test (d)	P=0.017N		
Fisher Exact Tests		P=0.218	P=0.041N
Tunica Vaginalis: Mesothelioma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (b)	2.8%	9.9%	6.7%
Terminal Rates (c)	1/36 (3%)	2/29 (7%)	0/0
Life Table Tests (d)	P=0.057	P=0.236	P=0.282
Incidental Tumor Tests (d)	P=0.320	P=0.287	P=0.748
Cochran-Armitage Trend Test (d)	P=0.610		
Fisher Exact Tests		P=0.309	P=0.753
All Sites: Mesothelioma			
Overall Rates (a)	1/50 (2%)	5/50 (10%)	3/50 (6%)
Adjusted Rates (b)	2.8%	15.1%	11.3%
Terminal Rates (c)	1/36 (3%)	3/29 (10%)	0/0
Life Table Tests (d)	P=0.005	P=0.072	P=0.052
Incidental Tumor Tests (d)	P=0.221	P=0.112	P=0.518
Cochran-Armitage Trend Test (d)	P=0.264		
Fisher Exact Tests		P=0.102	P=0.309
Zymbal Gland: Carcinoma			
Overall Rates (a)	1/50 (2%)	1/50 (2%)	13/50 (26%)
Adjusted Rates (b)	2.4%	3.4%	80.5%
Terminal Rates (c)	0/36 (0%)	1/29 (3%)	0/0
Life Table Tests (d)	P<0.001	P=0.715	P<0.001
Incidental Tumor Tests (d)	P=0.005	P=0.738N	P=0.062
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.753	P<0.001

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N). The results of the incidental tumor test are presented, but the test lacks sensitivity in this case due to the poor overlap in survival for the high dose and control groups.
(e) No P value is reported because no tumors were observed in the 1,000-ppm and control groups.
(f) Significance cannot be determined, since all tumors in controls were observed after the death of the last high dose animal and no tumors were observed in the high dose group.

TABLE E2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	500 ppm	1,000 ppm
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	0/50 (0%)	15/50 (30%)	10/50 (20%)
Adjusted Rates (b)	0.0%	37.8%	40.3%
Terminal Rates (c)	0/37 (0%)	11/35 (31%)	3/14 (21%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P=0.002	P<0.001	P=0.003
Cochran-Armitage Trend Test (d)	P=0.005		
Fisher Exact Tests		P<0.001	P<0.001
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	0/50 (0%)	16/50 (32%)	10/50 (20%)
Adjusted Rates (b)	0.0%	39.5%	40.3%
Terminal Rates (c)	0/37 (0%)	11/35 (31%)	3/14 (21%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P=0.003	P<0.001	P=0.003
Cochran-Armitage Trend Test (d)	P=0.006		
Fisher Exact Tests		P<0.001	P<0.001
Subcutaneous Tissue: Sarcoma or Fibrosarcoma			
Overall Rates (a)	1/50 (2%)	4/50 (8%)	2/50 (4%)
Adjusted Rates (b)	2.7%	9.6%	11.6%
Terminal Rates (c)	1/37 (3%)	0/35 (0%)	0/14 (0%)
Life Table Tests (d)	P=0.138	P=0.189	P=0.218
Incidental Tumor Tests (d)	P=0.486N	P=0.389	P=0.631
Cochran-Armitage Trend Test (d)	P=0.406		
Fisher Exact Tests		P=0.181	P=0.500
Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma			
Overall Rates (a)	1/50 (2%)	17/50 (34%)	12/50 (24%)
Adjusted Rates (b)	2.7%	40.9%	47.2%
Terminal Rates (c)	1/37 (3%)	11/35 (31%)	3/14 (21%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P=0.003	P<0.001	P<0.004
Cochran-Armitage Trend Test (d)	P=0.004		
Fisher Exact Tests		P<0.001	P<0.001
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	10/50 (20%)	11/50 (22%)	6/50 (12%)
Adjusted Rates (b)	22.7%	27.8%	22.8%
Terminal Rates (c)	4/37 (11%)	8/35 (23%)	2/14 (14%)
Life Table Tests (d)	P=0.430	P=0.496	P=0.567
Incidental Tumor Tests (d)	P=0.103N	P=0.576N	P=0.060N
Cochran-Armitage Trend Test (d)	P=0.181N		
Fisher Exact Tests		P=0.500	P=0.207N
Hematopoietic System: Leukemia			
Overall Rates (a)	11/50 (22%)	11/50 (22%)	6/50 (12%)
Adjusted Rates (b)	24.7%	27.8%	22.8%
Terminal Rates (c)	4/37 (11%)	8/35 (23%)	2/14 (14%)
Life Table Tests (d)	P=0.514	P=0.585	P=0.576N
Incidental Tumor Tests (d)	P=0.053N	P=0.438N	P=0.024N
Cochran-Armitage Trend Test (d)	P=0.124N		
Fisher Exact Tests		P=0.595	P=0.143N
Liver: Neoplastic Nodule			
Overall Rates (a)	1/50 (2%)	4/50 (8%)	3/50 (6%)
Adjusted Rates (b)	2.7%	11.0%	8.4%
Terminal Rates (c)	1/37 (3%)	3/35 (9%)	0/14 (0%)
Life Table Tests (d)	P=0.073	P=0.170	P=0.174
Incidental Tumor Tests (d)	P=0.250	P=0.211	P=0.375
Cochran-Armitage Trend Test (d)	P=0.252		
Fisher Exact Tests		P=0.181	P=0.309

TABLE E2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	500 ppm	1,000 ppm
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall Rates (a)	1/50 (2%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	2.7%	11.0%	14.9%
Terminal Rates (c)	1/37 (3%)	3/35 (9%)	1/14 (7%)
Life Table Tests (d)	P=0.025	P=0.170	P=0.062
Incidental Tumor Tests (d)	P=0.115	P=0.211	P=0.155
Cochran-Armitage Trend Test (d)	P=0.146		
Fisher Exact Tests		P=0.181	P=0.181
Pituitary: Adenoma			
Overall Rates (a)	26/50 (52%)	20/48 (42%)	21/49 (43%)
Adjusted Rates (b)	59.1%	47.0%	68.6%
Terminal Rates (c)	19/37 (51%)	13/35 (37%)	6/14 (43%)
Life Table Tests (d)	P=0.042	P=0.213N	P=0.030
Incidental Tumor Tests (d)	P=0.203N	P=0.049N	P=0.274N
Cochran-Armitage Trend Test (d)	P=0.207N		
Fisher Exact Tests		P=0.206N	P=0.239N
Pituitary: Adenoma or Carcinoma			
Overall Rates (a)	27/50 (54%)	22/48 (46%)	22/49 (45%)
Adjusted Rates (b)	61.3%	51.8%	72.5%
Terminal Rates (c)	20/37 (54%)	15/35 (43%)	7/14 (50%)
Life Table Tests (d)	P=0.027	P=0.274N	P=0.020
Incidental Tumor Tests (d)	P=0.279N	P=0.079N	P=0.348N
Cochran-Armitage Trend Test (d)	P=0.210N		
Fisher Exact Tests		P=0.272N	P=0.241N
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	1/49 (2%)
Adjusted Rates (b)	0.0%	8.1%	2.6%
Terminal Rates (c)	0/37 (0%)	2/35 (6%)	0/14 (0%)
Life Table Tests (d)	P=0.191	P=0.116	P=0.466
Incidental Tumor Tests (d)	P=0.372	P=0.158	P=0.602
Cochran-Armitage Trend Test (d)	P=0.372		
Fisher Exact Tests		P=0.121	P=0.495
Thyroid: Follicular Cell Adenoma			
Overall Rates (a)	0/47 (0%)	0/48 (0%)	4/50 (8%)
Adjusted Rates (b)	0.0%	0.0%	20.4%
Terminal Rates (c)	0/37 (0%)	0/33 (0%)	1/14 (7%)
Life Table Tests (d)	P=0.002	(e)	P=0.009
Incidental Tumor Tests (d)	P=0.025	(e)	P=0.135
Cochran-Armitage Trend Test (d)	P=0.017		
Fisher Exact Tests		(e)	P=0.066
Thyroid: Follicular Cell Adenoma or Carcinoma			
Overall Rates (a)	0/47 (0%)	2/48 (4%)	6/50 (12%)
Adjusted Rates (b)	0.0%	5.3%	29.2%
Terminal Rates (c)	0/37 (0%)	1/33 (3%)	2/14 (14%)
Life Table Tests (d)	P<0.001	P=0.232	P<0.001
Incidental Tumor Tests (d)	P=0.009	P=0.324	P=0.031
Cochran-Armitage Trend Test (d)	P=0.009		
Fisher Exact Tests		P=0.253	P=0.016
Thyroid: C-Cell Adenoma			
Overall Rates (a)	1/47 (2%)	3/48 (6%)	1/50 (2%)
Adjusted Rates (b)	2.7%	8.2%	4.2%
Terminal Rates (c)	1/37 (3%)	2/33 (6%)	0/14 (0%)
Life Table Tests (d)	P=0.353	P=0.285	P=0.590
Incidental Tumor Tests (d)	P=0.590	P=0.353	P=0.770N
Cochran-Armitage Trend Test (d)	P=0.587N		
Fisher Exact Tests		P=0.316	P=0.737N

TABLE E2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	500 ppm	1,000 ppm
Thyroid: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	2/47 (4%)	5/48 (10%)	2/50 (4%)
Adjusted Rates (b)	5.4%	14.1%	11.0%
Terminal Rates (c)	2/37 (5%)	4/33 (12%)	1/14 (7%)
Life Table Tests (d)	P=0.212	P=0.185	P=0.369
Incidental Tumor Tests (d)	P=0.364	P=0.226	P=0.544
Cochran-Armitage Trend Test (d)	P=0.553N		
Fisher Exact Tests		P=0.226	P=0.668N
Mammary Gland: Adenoma			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (b)	0.0%	7.9%	4.3%
Terminal Rates (c)	0/37 (0%)	2/35 (6%)	0/14 (0%)
Life Table Tests (d)	P=0.178	P=0.121	P=0.390
Incidental Tumor Tests (d)	P=0.385	P=0.158	P=0.707
Cochran-Armitage Trend Test (d)	P=0.378		
Fisher Exact Tests		P=0.121	P=0.500
Mammary Gland: Adenocarcinoma			
Overall Rates (a)	2/50 (4%)	2/50 (4%)	5/50 (10%)
Adjusted Rates (b)	5.4%	5.7%	23.2%
Terminal Rates (c)	2/37 (5%)	2/35 (6%)	2/14 (14%)
Life Table Tests (d)	P=0.020	P=0.675	P=0.035
Incidental Tumor Tests (d)	P=0.073	P=0.675	P=0.121
Cochran-Armitage Trend Test (d)	P=0.146		
Fisher Exact Tests		P=0.691	P=0.218
Mammary Gland: Adenoma or Adenocarcinoma			
Overall Rates (a)	2/50 (4%)	4/50 (8%)	6/50 (12%)
Adjusted Rates (b)	5.4%	10.7%	26.5%
Terminal Rates (c)	2/37 (5%)	3/35 (9%)	2/14 (14%)
Life Table Tests (d)	P=0.008	P=0.322	P=0.015
Incidental Tumor Tests (d)	P=0.062	P=0.372	P=0.095
Cochran-Armitage Trend Test (d)	P=0.099		
Fisher Exact Tests		P=0.339	P=0.134
Mammary Gland: Fibroadenoma			
Overall Rates (a)	22/50 (44%)	31/50 (62%)	29/50 (58%)
Adjusted Rates (b)	49.7%	73.4%	96.2%
Terminal Rates (c)	15/37 (41%)	24/35 (69%)	13/14 (93%)
Life Table Tests (d)	P<0.001	P=0.061	P<0.001
Incidental Tumor Tests (d)	P=0.006	P=0.091	P=0.023
Cochran-Armitage Trend Test (d)	P=0.096		
Fisher Exact Tests		P=0.054	P=0.115
Mammary Gland: Adenoma, Fibroadenoma, or Adenocarcinoma			
Overall Rates (a)	23/50 (46%)	32/50 (64%)	32/50 (64%)
Adjusted Rates (b)	52.0%	75.8%	96.6%
Terminal Rates (c)	16/37 (43%)	25/35 (71%)	13/14 (93%)
Life Table Tests (d)	P<0.001	P=0.059	P<0.001
Incidental Tumor Tests (d)	P=0.002	P=0.088	P=0.010
Cochran-Armitage Trend Test (d)	P=0.043		
Fisher Exact Tests		P=0.054	P=0.054
Clitoral Gland: Adenoma			
Overall Rates (a)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted Rates (b)	5.4%	0.0%	18.8%
Terminal Rates (c)	2/37 (5%)	0/35 (0%)	2/14 (14%)
Life Table Tests (d)	P=0.133	P=0.251N	P=0.136
Incidental Tumor Tests (d)	P=0.215	P=0.251N	P=0.247
Cochran-Armitage Trend Test (d)	P=0.390		
Fisher Exact Tests		P=0.247N	P=0.500

TABLE E2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	500 ppm	1,000 ppm
Clitoral Gland: Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	1/50 (2%)	5/50 (10%)
Adjusted Rates (b)	10.8%	2.5%	22.1%
Terminal Rates (c)	4/37 (11%)	0/35 (0%)	2/14 (14%)
Life Table Tests (d)	P=0.137	P=0.195N	P=0.126
Incidental Tumor Tests (d)	P=0.501	P=0.149N	P=0.382
Cochran-Armitage Trend Test (d)	P=0.421		
Fisher Exact Tests		P=0.181N	P=0.500
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	15/50 (30%)	14/50 (28%)	10/49 (20%)
Adjusted Rates (b)	36.9%	35.3%	41.4%
Terminal Rates (c)	12/37 (32%)	10/35 (29%)	4/14 (29%)
Life Table Tests (d)	P=0.225	P=0.541N	P=0.266
Incidental Tumor Tests (d)	P=0.303N	P=0.479N	P=0.426N
Cochran-Armitage Trend Test (d)	P=0.166N		
Fisher Exact Tests		P=0.500N	P=0.193N
Uterus: Endometrial Stromal Sarcoma			
Overall Rates (a)	1/50 (2%)	5/50 (10%)	6/49 (12%)
Adjusted Rates (b)	2.7%	13.3%	22.6%
Terminal Rates (c)	1/37 (3%)	4/35 (11%)	1/14 (7%)
Life Table Tests (d)	P=0.004	P=0.099	P=0.010
Incidental Tumor Tests (d)	P=0.058	P=0.120	P=0.136
Cochran-Armitage Trend Test (d)	P=0.045		
Fisher Exact Tests		P=0.102	P=0.053
Uterus: Endometrial Stromal Polyp or Sarcoma			
Overall Rates (a)	16/50 (32%)	18/50 (36%)	16/49 (33%)
Adjusted Rates (b)	39.4%	44.3%	56.2%
Terminal Rates (c)	13/37 (35%)	13/35 (37%)	5/14 (36%)
Life Table Tests (d)	P=0.014	P=0.379	P=0.024
Incidental Tumor Tests (d)	P=0.403	P=0.456	P=0.429
Cochran-Armitage Trend Test (d)	P=0.514		
Fisher Exact Tests		P=0.416	P=0.558
Zymbal Gland: Carcinoma			
Overall Rates (a)	0/50 (0%)	2/50 (4%)	7/50 (14%)
Adjusted Rates (b)	0.0%	4.0%	24.1%
Terminal Rates (c)	0/37 (0%)	0/35 (0%)	1/14 (7%)
Life Table Tests (d)	P<0.001	P=0.261	P=0.002
Incidental Tumor Tests (d)	P=0.056	P=0.187	P=0.059
Cochran-Armitage Trend Test (d)	P=0.003		
Fisher Exact Tests		P=0.247	P=0.006

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) No P value is reported because no tumors were observed in the 500-ppm and control groups.

TABLE E3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	500 ppm	1,000 ppm
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	7/50 (14%)	3/50 (6%)	6/50 (12%)
Adjusted Rates (b)	16.3%	8.8%	16.7%
Terminal Rates (c)	6/42 (14%)	2/32 (6%)	6/36 (17%)
Life Table Tests (d)	P=0.545N	P=0.285N	P=0.616
Incidental Tumor Tests (d)	P=0.476N	P=0.125N	P=0.551N
Cochran-Armitage Trend Test (d)	P=0.436N		
Fisher Exact Tests		P=0.159N	P=0.500N
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	4/50 (8%)	4/50 (8%)	2/50 (4%)
Adjusted Rates (b)	9.5%	12.5%	5.6%
Terminal Rates (c)	4/42 (10%)	4/32 (13%)	2/36 (6%)
Life Table Tests (d)	P=0.354N	P=0.488	P=0.410N
Incidental Tumor Tests (d)	P=0.354N	P=0.488	P=0.410N
Cochran-Armitage Trend Test (d)	P=0.274N		
Fisher Exact Tests		P=0.643N	P=0.339N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	11/50 (22%)	7/50 (14%)	8/50 (16%)
Adjusted Rates (b)	25.6%	20.9%	22.2%
Terminal Rates (c)	10/42 (24%)	6/32 (19%)	8/36 (22%)
Life Table Tests (d)	P=0.388N	P=0.434N	P=0.446N
Incidental Tumor Tests (d)	P=0.331N	P=0.276N	P=0.386N
Cochran-Armitage Trend Test (d)	P=0.254N		
Fisher Exact Tests		P=0.218N	P=0.306N
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	4/50 (8%)
Adjusted Rates (b)	6.7%	2.7%	9.4%
Terminal Rates (c)	2/42 (5%)	0/32 (0%)	2/36 (6%)
Life Table Tests (d)	P=0.364	P=0.388N	P=0.440
Incidental Tumor Tests (d)	P=0.595N	P=0.245N	P=0.556N
Cochran-Armitage Trend Test (d)	P=0.412		
Fisher Exact Tests		P=0.309N	P=0.500
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	2/50 (4%)	5/50 (10%)	3/50 (6%)
Adjusted Rates (b)	4.5%	13.2%	7.3%
Terminal Rates (c)	1/42 (2%)	1/32 (3%)	1/36 (3%)
Life Table Tests (d)	P=0.359	P=0.146	P=0.446
Incidental Tumor Tests (d)	P=0.533	P=0.387	P=0.600
Cochran-Armitage Trend Test (d)	P=0.421		
Fisher Exact Tests		P=0.218	P=0.500
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	4/50 (8%)
Adjusted Rates (b)	0.0%	9.4%	10.0%
Terminal Rates (c)	0/42 (0%)	3/32 (9%)	2/36 (6%)
Life Table Tests (d)	P=0.039	P=0.078	P=0.051
Incidental Tumor Tests (d)	P=0.047	P=0.078	P=0.100
Cochran-Armitage Trend Test (d)	P=0.049		
Fisher Exact Tests		P=0.121	P=0.059
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	7/50 (14%)	9/50 (18%)	11/50 (22%)
Adjusted Rates (b)	15.6%	23.7%	24.9%
Terminal Rates (c)	5/42 (12%)	4/32 (13%)	5/36 (14%)
Life Table Tests (d)	P=0.131	P=0.228	P=0.158
Incidental Tumor Tests (d)	P=0.301	P=0.494	P=0.459
Cochran-Armitage Trend Test (d)	P=0.181		
Fisher Exact Tests		P=0.393	P=0.218

TABLE E3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	500 ppm	1,000 ppm
Liver: Hepatocellular Adenoma			
Overall Rates (a)	22/50 (44%)	21/50 (42%)	17/50 (34%)
Adjusted Rates (b)	48.7%	55.9%	44.7%
Terminal Rates (c)	19/42 (45%)	16/32 (50%)	15/36 (42%)
Life Table Tests (d)	P=0.395N	P=0.260	P=0.413N
Incidental Tumor Tests (d)	P=0.264N	P=0.532	P=0.239N
Cochran-Armitage Trend Test (d)	P=0.179N		
Fisher Exact Tests		P=0.500N	P=0.206N
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	10/50 (20%)	20/50 (40%)	27/50 (54%)
Adjusted Rates (b)	23.1%	49.0%	62.5%
Terminal Rates (c)	9/42 (21%)	12/32 (38%)	20/36 (56%)
Life Table Tests (d)	P<0.001	P=0.005	P<0.001
Incidental Tumor Tests (d)	P<0.001	P=0.017	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.024	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	29/50 (58%)	37/50 (74%)	41/50 (82%)
Adjusted Rates (b)	62.8%	83.8%	91.1%
Terminal Rates (c)	25/42 (60%)	25/32 (78%)	32/36 (89%)
Life Table Tests (d)	P=0.001	P=0.004	P=0.001
Incidental Tumor Tests (d)	P<0.001	P=0.035	P=0.002
Cochran-Armitage Trend Test (d)	P=0.005		
Fisher Exact Tests		P=0.069	P=0.008
Thyroid: Follicular Cell Adenoma			
Overall Rates (a)	1/50 (2%)	1/48 (2%)	3/49 (6%)
Adjusted Rates (b)	2.4%	3.2%	7.3%
Terminal Rates (c)	1/42 (2%)	1/31 (3%)	1/36 (3%)
Life Table Tests (d)	P=0.175	P=0.693	P=0.262
Incidental Tumor Tests (d)	P=0.192	P=0.693	P=0.315
Cochran-Armitage Trend Test (d)	P=0.198		
Fisher Exact Tests		P=0.742	P=0.301
Harderian Gland: Adenoma			
Overall Rates (a)	3/50 (6%)	5/50 (10%)	3/50 (6%)
Adjusted Rates (b)	7.1%	14.4%	8.3%
Terminal Rates (c)	3/42 (7%)	4/32 (13%)	3/36 (8%)
Life Table Tests (d)	P=0.489	P=0.231	P=0.590
Incidental Tumor Tests (d)	P=0.507	P=0.293	P=0.590
Cochran-Armitage Trend Test (d)	P=0.576		
Fisher Exact Tests		P=0.357	P=0.661
Harderian Gland: Adenoma or Cystadenoma			
Overall Rates (a)	4/50 (8%)	6/50 (12%)	4/50 (8%)
Adjusted Rates (b)	9.5%	17.5%	11.1%
Terminal Rates (c)	4/42 (10%)	5/32 (16%)	4/36 (11%)
Life Table Tests (d)	P=0.468	P=0.224	P=0.557
Incidental Tumor Tests (d)	P=0.484	P=0.279	P=0.557
Cochran-Armitage Trend Test (d)	P=0.568		
Fisher Exact Tests		P=0.370	P=0.643

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

TABLE E4. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control	500 ppm	1,000 ppm
Subcutaneous Tissue: Sarcoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted Rates (b)	8.4%	6.1%	0.0%
Terminal Rates (c)	1/31 (3%)	0/12 (0%)	0/6 (0%)
Life Table Tests (d)	P=0.266N	P=0.626	P=0.384N
Incidental Tumor Tests (d)	P=0.042N	P=0.335N	P=0.103N
Cochran-Armitage Trend Test (d)	P=0.082N		
Fisher Exact Tests		P=0.500N	P=0.121N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	0/50 (0%)	2/49 (4%)	4/47 (9%)
Adjusted Rates (b)	0.0%	11.4%	25.2%
Terminal Rates (c)	0/31 (0%)	1/12 (8%)	1/6 (17%)
Life Table Tests (d)	P=0.004	P=0.112	P=0.011
Incidental Tumor Tests (d)	P=0.020	P=0.211	P=0.083
Cochran-Armitage Trend Test (d)	P=0.032		
Fisher Exact Tests		P=0.242	P=0.051
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	0/50 (0%)	2/49 (4%)	5/47 (11%)
Adjusted Rates (b)	0.0%	11.4%	27.6%
Terminal Rates (c)	0/31 (0%)	1/12 (8%)	1/6 (17%)
Life Table Tests (d)	P=0.002	P=0.112	P=0.005
Incidental Tumor Tests (d)	P=0.009	P=0.211	P=0.055
Cochran-Armitage Trend Test (d)	P=0.014		
Fisher Exact Tests		P=0.242	P=0.024
Hematopoietic System: Malignant Lymphoma, Undifferentiated Type			
Overall Rates (a)	6/50 (12%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	17.9%	8.3%	6.2%
Terminal Rates (c)	4/31 (13%)	1/12 (8%)	0/6 (0%)
Life Table Tests (d)	P=0.343N	P=0.303N	P=0.566N
Incidental Tumor Tests (d)	P=0.119N	P=0.210N	P=0.181N
Cochran-Armitage Trend Test (d)	P=0.023N		
Fisher Exact Tests		P=0.056N	P=0.056N
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	0/50 (0%)	5/50 (10%)	1/50 (2%)
Adjusted Rates (b)	0.0%	25.5%	7.1%
Terminal Rates (c)	0/31 (0%)	2/12 (17%)	0/6 (0%)
Life Table Tests (d)	P=0.095	P=0.005	P=0.307
Incidental Tumor Tests (d)	P=0.392	P=0.028	P=0.588
Cochran-Armitage Trend Test (d)	P=0.399		
Fisher Exact Tests		P=0.028	P=0.500
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	8/50 (16%)
Adjusted Rates (b)	2.2%	8.5%	30.5%
Terminal Rates (c)	0/31 (0%)	0/12 (0%)	0/6 (0%)
Life Table Tests (d)	P=0.002	P=0.213	P=0.003
Incidental Tumor Tests (d)	P=0.176	P=0.714	P=0.284
Cochran-Armitage Trend Test (d)	P=0.008		
Fisher Exact Tests		P=0.309	P=0.015
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	17/50 (34%)	24/50 (48%)	25/50 (50%)
Adjusted Rates (b)	43.3%	74.1%	77.9%
Terminal Rates (c)	10/31 (32%)	6/12 (50%)	2/6 (33%)
Life Table Tests (d)	P<0.001	P<0.001	P=0.001
Incidental Tumor Tests (d)	P=0.160	P=0.171	P=0.245
Cochran-Armitage Trend Test (d)	P=0.065		
Fisher Exact Tests		P=0.111	P=0.078

TABLE E4. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	500 ppm	1,000 ppm
Circulatory System: Hemangioma or Angiosarcoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	9.7%	4.0%	3.4%
Terminal Rates (c)	3/31 (10%)	0/12 (0%)	0/6 (0%)
Life Table Tests (d)	P=0.581N	P=0.624N	P=0.689
Incidental Tumor Tests (d)	P=0.394N	P=0.479N	P=0.660N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Tests		P=0.309N	P=0.309N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	2/49 (4%)	18/50 (36%)	4/49 (8%)
Adjusted Rates (b)	6.5%	73.8%	22.6%
Terminal Rates (c)	2/31 (6%)	7/12 (58%)	1/6 (17%)
Life Table Tests (d)	P=0.004	P<0.001	P=0.063
Incidental Tumor Tests (d)	P=0.165	P<0.001	P=0.268
Cochran-Armitage Trend Test (d)	P=0.341		
Fisher Exact Tests		P<0.001	P=0.339
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	3/49 (6%)	19/50 (38%)	37/49 (76%)
Adjusted Rates (b)	9.2%	70.5%	97.1%
Terminal Rates (c)	2/31 (6%)	6/12 (50%)	5/6 (83%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	5/49 (10%)	35/50 (70%)	41/49 (84%)
Adjusted Rates (b)	15.5%	96.9%	100.0%
Terminal Rates (c)	4/31 (13%)	11/12 (92%)	6/6 (100%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
Pituitary: Adenoma			
Overall Rates (a)	10/38 (26%)	7/36 (19%)	2/29 (7%)
Adjusted Rates (b)	37.1%	45.0%	12.1%
Terminal Rates (c)	8/24 (33%)	4/11 (36%)	0/6 (0%)
Life Table Tests (d)	P=0.526N	P=0.297	P=0.470N
Incidental Tumor Tests (d)	P=0.178N	P=0.485	P=0.163N
Cochran-Armitage Trend Test (d)	P=0.032N		
Fisher Exact Tests		P=0.336N	P=0.039N
Adrenal: Pheochromocytoma			
Overall Rates (a)	1/48 (2%)	7/47 (15%)	7/45 (16%)
Adjusted Rates (b)	3.2%	32.4%	59.8%
Terminal Rates (c)	1/31 (3%)	2/12 (17%)	3/6 (50%)
Life Table Tests (d)	P<0.001	P=0.003	P<0.001
Incidental Tumor Tests (d)	P=0.006	P=0.030	P=0.003
Cochran-Armitage Trend Test (d)	P=0.025		
Fisher Exact Tests		P=0.027	P=0.024
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant			
Overall Rates (a)	1/48 (2%)	8/47 (17%)	8/45 (18%)
Adjusted Rates (b)	3.2%	37.2%	73.2%
Terminal Rates (c)	1/31 (3%)	2/12 (17%)	4/6 (67%)
Life Table Tests (d)	P<0.001	P=0.001	P<0.001
Incidental Tumor Tests (d)	P=0.002	P=0.016	P<0.001
Cochran-Armitage Trend Test (d)	P=0.015		
Fisher Exact Tests		P=0.014	P=0.012

TABLE E4. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (Continued)

	Control	500 ppm	1,000 ppm
Harderian Gland: Adenoma			
Overall Rates (a)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted Rates (b)	0.0%	0.0%	27.6%
Terminal Rates (c)	0/31 (0%)	0/12 (0%)	1/6 (17%)
Life Table Tests (d)	P=0.004	(e)	P=0.011
Incidental Tumor Tests (d)	P=0.021	(e)	P=0.088
Cochran-Armitage Trend Test (d)	P=0.037		
Fisher Exact Tests		(e)	P=0.121
Harderian Gland: Adenoma or Cystadenoma			
Overall Rates (a)	0/50 (0%)	0/50 (0%)	5/50 (10%)
Adjusted Rates (b)	0.0%	0.0%	44.3%
Terminal Rates (c)	0/31 (0%)	0/12 (0%)	2/6 (33%)
Life Table Tests (d)	P<0.001	(e)	P<0.001
Incidental Tumor Tests (d)	P=0.001	(e)	P=0.011
Cochran-Armitage Trend Test (d)	P=0.006		
Fisher Exact Tests		(e)	P=0.028
Harderian Gland: Adenoma, Cystadenoma, or Carcinoma			
Overall Rates (a)	1/50 (2%)	0/50 (0%)	5/50 (10%)
Adjusted Rates (b)	2.5%	0.0%	44.3%
Terminal Rates (c)	0/31 (0%)	0/12 (0%)	2/6 (33%)
Life Table Tests (d)	P=0.002	P=0.628N	P=0.004
Incidental Tumor Tests (d)	P=0.019	P=0.479N	P=0.070
Cochran-Armitage Trend Test (d)	P=0.037		
Fisher Exact Tests		P=0.500N	P=0.102

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) No P value is reported because no tumors were observed in the 500-ppm and control groups.

APPENDIX F

HISTORICAL INCIDENCES OF TUMORS IN F344/N RATS AND B6C3F$_1$ MICE RECEIVING NO TREATMENT

TABLE F1. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls			
	Papilloma NOS	Squamous Cell Papilloma	Squamous Cell Carcinoma	Papilloma or Carcinoma
Historical Incidence at Mason Research Institute				
4,4'-Methylenedianiline · 2HCl	0/50	0/50	1/50	1/50
Monuron	0/50	0/50	1/50	1/50
8-Hydroxyquinoline	0/50	0/50	1/50	1/50
Di(2-ethylhexyl)phthalate	0/50	1/50	0/50	1/50
Di(2-ethylhexyl)adipate	0/49	1/49	0/49	1/49
Guar gum	0/50	0/50	0/50	0/50
Locust bean gum	0/50	1/50	0/50	1/50
Gum arabic	0/50	1/50	0/50	1/50
Agar	0/50	1/50	1/50	2/50
Tara gum	0/50	1/50	2/50	3/50
2,6-Toluenediamine · 2HCl	0/50	0/50	1/50	1/50
4,4'-Oxydianiline	0/50	2/50	0/50	2/50
2-Biphenylamine · HCl	0/50	1/50	0/50	1/50
Cinnamyl anthranilate	0/50	0/50	1/50	1/50
TOTAL	0/699 (0.0%)	9/699 (1.3%)	8/699 (1.1%)	17/699 (2.4%)
SD (b)	0.00%	1.27%	1.29%	1.40%
Range (c)				
High	0/50	2/50	2/50	3/50
Low	0/50	0/50	0/50	0/50
Overall Historical Incidence				
TOTAL	5/2,372 (0.2%)	22/2,372 (0.9%)	17/2,372 (0.7%)	44/2,372 (1.9%)
SD (b)	0.86%	1.46%	1.14%	1.83%
Range (c)				
High	2/50	2/40	2/50	3/50
Low	0/90	0/52	0/90	0/52

TABLE F1. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a) (Continued)

Study	Incidence in Controls (Continued)			
	Basal Cell Tumor	Basal Cell Carcinoma	Tricho-epithelioma	Sebaceous Adenoma
Historical Incidence at Mason Research Institute (Continued)				
4,4'-Methylenedianiline · 2HCl	0/50	0/50	0/50	0/50
Monuron	0/50	0/50	1/50	0/50
8-Hydroxyquinoline	0/50	1/50	0/50	0/50
Di(2-ethylhexyl)phthalate	0/50	0/50	0/50	1/50
Di(2-ethylhexyl)adipate	0/49	0/49	0/49	0/49
Guar gum	0/50	0/50	0/50	0/50
Locust bean gum	0/50	0/50	0/50	0/50
Gum arabic	0/50	0/50	0/50	0/50
Agar	0/50	0/50	0/50	0/50
Tara gum	0/50	1/50	0/50	0/50
2,6-Toluenediamine · 2HCl	0/50	0/50	0/50	0/50
4,4'-Oxydianiline	0/50	3/50	0/50	0/50
2-Biphenylamine · HCl	0/50	0/50	0/50	0/50
Cinnamyl anthranilate	0/50	0/50	0/50	0/50
TOTAL	0/699 (0.0%)	5/699 (0.7%)	1/699 (0.1%)	1/699 (0.1%)
SD (b)	0.00%	1.68%	0.53%	0.53%
Range (c)				
High	0/50	3/50	1/50	1/50
Low	0/50	0/50	0/50	0/50
Overall Historical Incidence				
TOTAL	7/2,372 (0.3%)	14/2,372 (0.6%)	4/2,372 (0.2%)	(d) 3/2,372 (0.1%)
SD (b)	0.84%	1.33%	0.57%	0.43%
Range (c)				
High	2/50	3/50	1/49	1/50
Low	0/90	0/90	0/90	0/52

TABLE F1. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a) (Continued)

Study	Incidence in Controls (Continued)			
	Fibroma	Fibrosarcoma	Fibroma or Fibrosarcoma	Sarcoma NOS
Historical Incidence at Mason Research Institute (Continued)				
4,4'-Methylenedianiline · 2HCl	5/50	0/50	5/50	0/50
Monuron	1/50	0/50	1/50	0/50
8-Hydroxyquinoline	2/50	0/50	2/50	2/50
Di(2-ethylhexyl)phthalate	1/50	1/50	2/50	0/50
Di(2-ethylhexyl)adipate	4/49	0/49	4/49	0/49
Guar gum	0/50	1/50	1/50	1/50
Locust bean gum	0/50	1/50	1/50	0/50
Gum arabic	2/50	0/50	2/50	0/50
Agar	2/50	1/50	3/50	0/50
Tara gum	1/50	0/50	1/50	1/50
2,6-Toluenediamine · 2HCl	0/50	0/50	0/50	0/50
4,4'-Oxydianiline	1/50	0/50	1/50	1/50
2-Biphenylamine · HCl	6/50	0/50	6/50	0/50
Cinnamyl anthranilate	0/50	0/50	0/50	0/50
TOTAL	25/699 (3.6%)	4/699 (0.6%)	29/699 (4.1%)	5/699 (0.7%)
SD (b)	3.87%	0.94%	3.65%	1.27%
Range (c)				
High	6/50	1/50	6/50	2/50
Low	0/50	0/50	0/50	0/50
Overall Historical Incidence				
TOTAL	(e) 105/2,372 (4.4%)	20/2,372 (0.8%)	124/2,372 (5.2%)	8/2,372 (0.3%)
SD (b)	3.10%	1.23%	3.30%	0.87%
Range (c)				
High	6/50	2/50	6/49	2/50
Low	0/50	0/52	0/50	0/90

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) One sebaceous adenocarcinoma and one adenocarcinoma, NOS, also were observed.
(e) One fibroadenoma was also observed.

TABLE F2. HISTORICAL INCIDENCE OF LIVER TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Neoplastic Nodule	Hepatocellular Carcinoma	Neoplastic Nodule or Hepatocellular Carcinoma
Historical Incidence at Mason Research Institute			
4,4'-Methylenedianiline · 2HCl	1/50	0/50	1/50
Monuron	1/50	0/50	1/50
8-Hydroxyquinoline	6/49	1/49	7/49
Di(2-ethylhexyl)phthalate	2/50	1/50	3/50
Di(2-ethylhexyl)adipate	2/49	0/49	2/49
Guar gum	2/50	1/50	(b) 3/50
Locust bean gum	0/50	1/50	1/50
Gum arabic	3/49	1/49	4/49
Agar	0/50	0/50	0/50
Tara gum	1/49	0/49	1/49
2,6-Toluenediamine · 2 HCl	0/50	0/50	0/50
4,4'-Oxydianiline	1/50	0/50	1/50
2-Biphenylamine · HCl	0/49	0/49	0/49
Cinnamyl anthranilate	1/48	0/48	1/48
TOTAL	20/693 (2.9%)	5/693 (0.7%)	25/693 (3.6%)
SD (c)	3.27%	1.0%	3.93%
Range (d)			
High	6/49	1/49	7/49
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	(e) 90/2,358 (3.8%)	20/2,358 (0.8%)	(e) 110/2,358 (4.7%)
SD (c)	4.47%	1.16%	5.06%
Range (d)			
High	(f) 12/52	2/49	(g) 14/52
Low	0/50	0/90	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) One bile duct carcinoma was also observed.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes one group of 12/52 diagnosed as hepatocellular adenoma
(f) Diagnosed as hepatocellular adenoma; second highest: two groups of 6/49
(g) Second highest: two groups of 7/49

TABLE F3. HISTORICAL INCIDENCE OF LEUKEMIA IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence of Leukemia in Controls
Historical Incidence at Mason Research Institute	
4,4'-Methylenedianiline · 2HCl	12/50
Monuron	5/50
8-Hydroxyquinoline	17/50
Di(2-ethylhexyl)phthalate	13/50
Di(2-ethylhexyl)adipate	9/49
Guar gum	13/50
Locust bean gum	21/50
Gum arabic	10/50
Agar	9/50
Tara gum	14/50
2,6-Toluenediamine · 2 HCl	9/50
4,4'-Oxydianiline	23/50
2-Biphenylamine · HCl	15/50
Cinnamyl anthranilate	(b) 0/50
TOTAL	170/699 (24.3%)
SD (c)	11.96%
Range (d)	
High	23/50
Low	(e) 0/50
Overall Historical Incidence	
TOTAL	650/2,372 (27.4%)
SD (c)	10.67%
Range (d)	
High	23/50
Low	(e) 0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) The incidence of malignant lymphoma in this group was 7/50 and possibly represents a difference in nomenclature.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Second lowest: 5/50

TABLE F4. HISTORICAL INCIDENCE OF THYROID GLAND FOLLICULAR CELL TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Mason Research Institute			
4,4'-Methylenedianiline · 2HCl	1/49	0/49	1/49
Monuron	0/49	0/49	0/49
8-Hydroxyquinoline	0/50	0/50	0/50
Di(2-ethylhexyl)phthalate	0/48	1/48	1/48
Di(2-ethylhexyl)adipate	0/49	0/49	0/49
Guar gum	0/50	0/50	0/50
Locust bean gum	0/49	0/49	0/49
Gum arabic	0/47	(b) 0/47	(b) 0/47
Agar	0/49	0/49	0/49
Tara gum	0/45	0/45	0/45
2,6-Toluenediamine · 2HCl	0/44	0/44	0/44
4,4'-Oxydianiline	1/46	0/46	1/46
2-Biphenylamine · HCl	0/47	0/47	0/47
Cinnamyl anthranilate	0/42	1/42	1/42
TOTAL	2/664 (0.3%)	2/664 (0.3%)	4/664 (0.6%)
SD (c)	0.77%	0.81%	1.02%
Range(d)			
High	1/46	1/42	1/42
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	(e) 22/2,282 (1.0%)	(b) 16/2,282 (0.7%)	(e) 38/2,282 (1.7%)
SD (c)	1.32%	1.37%	2.03%
Range(d)			
High	2/44	3/45	4/45
Low	0/50	0/52	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) One papillary adenocarcinoma of the thyroid follicle was also observed.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes one cystadenoma, NOS of the thyroid gland follicle.

TABLE F5. HISTORICAL INCIDENCE OF LUNG TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Alveolar/Bronchiolar Adenoma	Alveolar/Bronchiolar Carcinoma	Alveolar/Bronchiolar Adenoma or Carcinoma
Historical Incidence at Mason Research Institute			
4,4'-Methylenedianiline·2HCl	2/50	0/50	2/50
Monuron	1/50	0/50	1/50
8-Hydroxyquinoline	0/50	0/50	0/50
Di(2-ethylhexyl)phthalate	1/50	0/50	1/50
Di(2-ethylhexyl)adipate	0/49	0/49	0/49
Guar gum	0/50	1/50	1/50
Locust bean gum	0/50	0/50	0/50
Gum arabic	0/50	0/50	0/50
Agar	0/50	0/50	0/50
Tara gum	2/50	0/50	2/50
2,6-Toluenediamine·2HCl	3/49	0/49	3/49
4,4'-Oxydianiline	1/50	0/50	1/50
2-Biphenylamine·HCl	2/50	0/50	2/50
Cinnamyl anthranilate	0/48	0/48	0/48
TOTAL	12/696 (1.7%)	1/696 (0.1%)	13/696 (1.9%)
SD (b)	2.07%	0.53%	2.01%
Range (c)			
High	3/49	1/50	3/49
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	36/2,357 (1.5%)	23/2,357 (1.0%)	57/2,357 (2.4%)
SD (b)	2.05%	1.71%	2.35%
Range (c)			
High	3/47	3/50	4/49
Low	0/89	0/50	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE F6. HISTORICAL INCIDENCE OF ZYMBAL GLAND CARCINOMAS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence of Carcinoma in Controls
Historical Incidence at Mason Research Institute	
4,4'-Methylenedianiline · 2HCl	0/50
Monuron	0/50
8-Hydroxyquinoline	1/50
Di(2-ethylhexyl)phthalate	0/50
Di(2-ethylhexyl)adipate	0/49
Guar gum	1/50
Locust bean gum	0/50
Gum arabic	0/50
Agar	0/50
Tara gum	0/50
2,6-Toluenediamine · 2HCl	0/50
4,4'-Oxydianiline	0/50
2-Biphenylamine · HCl	1/50
Cinnamyl anthranilate	0/50
TOTAL	(b) 3/699 (0.4%)
SD (c)	0.85%
Range (d)	
High	1/50
Low	0/50
Overall Historical Incidence	
TOTAL	(e) 11/2,372 (0.5%)
SD (c)	1.12%
Range (d)	
High	3/50
Low	0/90

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Includes two squamous cell carcinomas and one ceruminous carcinoma
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes nine squamous cell carcinomas, one carcinoma, NOS and one ceruminous carcinoma. The only other Zymbal gland tumor observed was one carcinosarcoma.

TABLE F7. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Fibroma	Fibrosarcoma	Fibroma or Fibrosarcoma
Historical Incidence at Mason Research Institute			
4,4'-Methylenedianiline · 2HCl	1/50	1/50	2/50
Monuron	0/50	0/50	0/50
8-Hydroxyquinoline	1/50	1/50	2/50
Butyl benzyl phthalate	1/49	0/49	1/49
Di(2-ethylhexyl)phthalate	0/50	0/50	0/50
Di(2-ethylhexyl)adipate	0/50	1/50	1/50
Guar gum	0/50	0/50	0/50
Locust bean gum	1/50	1/50	2/50
Gum Arabic	0/50	0/50	0/50
Agar	2/50	0/50	2/50
Tara gum	2/50	0/50	2/50
2,6-Toluenediamine · 2HCl	1/50	0/50	1/50
4,4'-Oxydianiline	0/50	0/50	0/50
2-Biphenylamine · HCl	1/50	0/50	1/50
Cinnamyl anthranilate	1/48	0/48	1/48
TOTAL	11/747 (1.5%)	4/747 (0.5%)	15/747 (2.0%)
SD (b)	1.41%	0.92%	1.69%
Range (c)			
High	2/50	1/50	2/50
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	30/2,422 (1.2%)	12/2,422 (0.5%)	42/2,422 (1.7%)
SD (b)	1.36%	0.88%	1.70%
Range (c)			
High	2/50	1/49	3/50
Low	0/50	0/88	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE F8. HISTORICAL INCIDENCE OF LIVER TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Neoplastic Nodule	Hepatocellular Carcinoma	Neoplastic Nodule or Hepatocellular Carcinoma
Historical Incidence at Mason Research Institute			
4,4'-Methylenedianiline · 2HCl	4/50	0/50	4/50
Monuron	4/50	0/50	4/50
8-Hydroxyquinoline	3/50	1/50	4/50
Butyl benzyl phthalate	1/49	0/49	1/49
Di(2-ethylhexyl)phthalate	0/50	0/50	0/50
Di(2-ethylhexyl)adipate	0/49	0/49	0/49
Guar gum	2/49	0/49	2/49
Locust bean gum	0/50	0/50	0/50
Gum Arabic	3/49	0/49	3/49
Agar	0/50	0/50	0/50
Tara gum	2/49	0/49	2/49
2,6-Toluenediamine · 2HCl	0/50	0/50	0/50
4,4'-Oxydianiline	3/50	0/50	3/50
2-Biphenylamine · HCl	1/50	0/50	1/50
Cinnamyl anthranilate	2/46	0/46	(b) 2/46
TOTAL	(b) 25/741 (3.4%)	1/741 (0.1%)	(b) 26/741 (3.5%)
SD (c)	3.01%	0.52%	3.17%
Range (d)			
High	4/50	1/50	4/50
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	(e) 85/2,408 (3.5%)	5/2,408 (0.2%)	(e) 89/2,408 (3.7%)
SD (c)	4.58%	0.74%	4.88%
Range (d)			
High	(f) 14/52	2/50	(g) 15/52
Low	0/50	0/88	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Includes one hepatocellular adenoma
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes 16 tumors (14/52 in one group) designated hepatocellular adenoma
(f) Diagnosed as hepatocellular adenoma; second highest: 6/50
(g) Includes 14/52 diagnosed as hepatocellular adenoma; second highest: 6/50

TABLE F9. HISTORICAL INCIDENCE OF THYROID GLAND FOLLICULAR CELL TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Mason Research Institute			
4,4'-Methylenedianiline · 2HCl	0/47	0/47	0/47
Monuron	0/49	0/49	0/49
8-Hydroxyquinoline	0/48	0/48	0/48
Butyl benzyl phthalate	0/47	0/47	0/47
Di(2-ethylhexyl)phthalate	0/48	0/48	0/48
Di(2-ethylhexyl)adipate	0/50	0/50	(b) 0/50
Guar gum	0/48	0/48	0/48
Locust bean gum	0/50	0/50	0/50
Gum Arabic	(c) 0/49	1/49	1/49
Agar	0/49	1/49	1/49
Tara gum	0/46	0/46	0/46
2,6-Toluenediamine · 2HCl	0/49	0/49	0/49
4,4'-Oxydianiline	0/49	0/49	0/49
2-Biphenylamine · HCl	1/49	0/49	1/49
Cinnamyl anthranilate	0/46	0/46	0/46
TOTAL	1/724 (0.1%)	2/724 (0.3%)	3/724 (0.4%)
SD (d)	0.53%	0.72%	0.84%
Range (e)			
High	1/49	1/49	1/49
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	7/2,317 (0.3%)	8/2,317 (0.3%)	(f) 15/2,317 (0.6%)
SD (d)	0.71%	0.80%	0.97%
Range (e)			
High	1/42	1/39	1/39
Low	0/52	0/86	0/52

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) One papillary cystadenocarcinoma was observed.
(c) One papillary cystadenoma was observed.
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.
(f) One of each of the following tumors was also observed in the thyroid gland follicle: papillary adenoma, cystadenoma, NOS, papillary cystadenoma, NOS, papillary carcinoma, and papillary cystadenocarcinoma, NOS. The inclusion of these tumors would increase the high range to 2/42.

TABLE F10. HISTORICAL INCIDENCE OF MAMMARY GLAND TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Fibroadenoma	Adenocarcinoma, NOS	All Adenocarcinoma
Historical Incidence at Mason Research Institute			
4,4'-Methylenedianiline · 2HCl	10/50	4/50	4/50
Monuron	20/50	1/50	1/50
8-Hydroxyquinoline	19/50	0/50	0/50
Butyl benzyl phthalate	20/49	2/49	2/49
Di(2-ethylhexyl)phthalate	10/50	1/50	2/50
Di(2-ethylhexyl)adipate	13/50	1/50	1/50
Guar gum	20/50	2/50	2/50
Locust bean gum	16/50	1/50	1/50
Gum Arabic	14/50	0/50	0/50
Agar	14/50	3/50	3/50
Tara gum	13/50	0/50	0/50
2,6-Toluenediamine · 2HCl	11/50	0/50	0/50
4,4'-Oxydianiline	16/50	0/50	0/50
2-Biphenylamine · HCl	22/50	1/50	1/50
Cinnamyl anthranilate	8/48	0/48	0/48
TOTAL	(b) 226/747 (30.3%)	16/747 (2.1%)	17/747 (2.3%)
SD (c)	8.73%	2.45%	2.50%
Range (d)			
High	22/50	4/50	4/50
Low	8/48	0/50	0/50
Overall Historical Incidence			
TOTAL	(e) 549/2,422 (22.7%)	42/2,422 (1.7%)	(f) 49/2,422 (2.0%)
SD (c)	10.39%	2.18%	2.34%
Range (d)			
High	22/50	4/50	4/49
Low	0/50	0/50	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Four adenomas, NOS, and one papillary cystadenoma also have been observed.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) One fibroma, 18 adenomas, NOS, 7 cystadenomas, 2 papillary cystadenomas, 4 cystfibroadenomas, and 1 acinar cell adenoma also have been observed.
(f) Includes one carcinoma, NOS

TABLE F11. HISTORICAL INCIDENCE OF UTERINE GLANDULAR TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	No. of Animals Examined	No. of Tumors in Controls	Site	Diagnosis
Historical Incidence at Mason Research Institute				
4,4'-Methylenedianiline·2HCl	48	1	Uterus, NOS	Papillary adenocarcinoma
4,4'-Oxydianiline	49	2	Uterus, NOS	Adenocarcinoma, NOS
All others	636	0		
TOTAL	733	3 (0.4%)		
Overall Historical Incidence (b)				
	2,370	1	Uterus, NOS	Carcinoma-in-situ, NOS
		6	Uterus, NOS	Adenocarcinoma, NOS
		2	Uterus, NOS	Papillary Adenocarcinoma
		1	Uterus/endometrium	Adenoma, NOS
		4	Uterus/endometrium	Adenocarcinoma, NOS
		1	Uterus/endometrium	Papillary Adenocarcinoma
TOTAL		15 (0.6%)		

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Greatest incidence observed in any control group: 2/45

TABLE F12. HISTORICAL INCIDENCE OF UTERINE ENDOMETRIAL TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls	
	Endometrial Stromal Polyp	Endometrial Stromal Sarcoma
Historical Incidence at Mason Research Institute		
4,4'-Methylenedianiline · 2HCl	11/48	3/48
Monuron	9/50	1/50
8-Hydroxyquinoline	11/49	0/49
Butyl benzyl phthalate	12/49	0/49
Di(2-ethylhexyl)phthalate	7/49	0/49
Di(2-ethylhexyl)adipate	11/50	1/50
Guar gum	17/49	0/49
Locust bean gum	12/50	0/50
Gum Arabic	14/49	1/49
Agar	17/50	0/50
Tara gum	6/47	0/47
2,6-Toluenediamine · 2HCl	9/48	1/48
4,4'-Oxydianiline	7/49	0/49
2-Biphenylamine · HCl	9/49	0/49
Cinnamyl anthranilate	2/47	0/47
TOTAL	154/733 (21.0%)	7/733 (1.0%)
SD (b)	8.02%	1.73%
Range (c)		
High	17/49	3/48
Low	2/47	0/50
Overall Historical Incidence		
TOTAL	429/2,370 (18.1%)	22/2,370 (0.9%)
SD (b)	8.10%	1.58%
Range (c)		
High	18/49	3/48
Low	2/47	0/87

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE F13. HISTORICAL INCIDENCE OF ZYMBAL GLAND CARCINOMAS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence of Carcinoma in Controls (b)
Historical Incidence at Mason Research Institute	
Monuron	1/50
2,6-Toluenediamine · 2HCl	1/50
All others	0/647
TOTAL	2/747 (0.3%)
SD (c)	0.70%
Range (d)	
High	1/50
Low	0/50
Overall Historical Incidence	
TOTAL	(e) 6/2,422 (0.2%)
SD (c)	0.67%
Range (d)	
High	(e) 1/50
Low	0/88

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Includes carcinoma, NOS, and squamous cell carcinoma. No ceruminous carcinomas were observed.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) One adenocarcinoma, NOS, and two adenosquamous carcinomas were also observed. The inclusion of these tumors would increase the high range to 2/50.

TABLE F14. HISTORICAL INCIDENCE OF KIDNEY TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	No. of Animals Examined	No. of Tumors in Controls	Site	Diagnosis
Historical Incidence at Mason Research Institute				
	742			
No kidney tumors were observed at this laboratory.				
Overall Historical Incidence				
	2,411	1	Kidney, NOS	Tubular cell adenoma
		3	Kidney, NOS	Tubular cell adenocarcinoma
TOTAL		4 (<1%)		

(a) Data as of March 16, 1983, for studies of at least 104 weeks

TABLE F15. HISTORICAL INCIDENCE OF URINARY BLADDER TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	No. of Animals Examined	No. of Tumors in Controls	Diagnosis
Historical Incidence at Mason Research Institute			
Di(2-ethylhexyl)adipate	49	1	Transitional cell papilloma
2-Biphenylamine · HCl	49	1	Transitional cell carcinoma
All others	630	0	
TOTAL	728	2 (<1%)	
Overall Historical Incidence			
	2,422	2	Papilloma, NOS
		2	Transitional cell papilloma
		1	Transitional cell carcinoma
TOTAL		5 (<1%)	

(a) Data as of March 16, 1983, for studies of at least 104 weeks

TABLE F16. HISTORICAL INCIDENCE OF LIVER TUMORS IN MALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence at Mason Research Institute			
4,4'-Methylenedianiline · 2HCl	7/49	10/49	17/49
Monuron	7/50	6/50	12/50
8-Hydroxyquinoline	9/50	5/50	14/50
Butyl benzyl phthalate	4/50	9/50	13/50
Di(2-ethylhexyl)phthalate	6/50	9/50	14/50
Di(2-ethylhexyl)adipate	6/50	7/50	13/50
Guar gum	1/50	15/50	16/50
Locust bean gum	6/50	15/50	18/50
Gum arabic	4/49	13/49	16/49
Agar	0/49	9/49	9/49
Tara gum	8/50	9/50	17/50
2,6-Toluenediamine · 2 HCl	7/50	14/50	21/50
4,4'-Oxydianiline	11/50	18/50	29/50
2-Biphenylamine · HCl	5/50	9/50	14/50
Cinnamyl anthranilate	8/48	6/48	14/48
TOTAL	89/745 (11.9%)	154/745 (20.7%)	237/745 (31.8%)
SD (b)	5.77%	7.71%	9.18%
Range (c)			
High	11/50	18/50	29/50
Low	0/49	5/50	9/49
Overall Historical Incidence			
TOTAL	242/2,386 (10.1%)	501/2,386 (21%)	730/2,386 (30.6%)
SD (b)	5%	7.25%	8.01%
Range (c)			
High	11/50	18/50	29/50
Low	0/49	3/52	5/52

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE F17. HISTORICAL INCIDENCE OF HEMATOPOIETIC SYSTEM TUMORS IN MALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls	
	Malignant Lymphoma	Lymphoma or Leukemia
Historical Incidence at Mason Research Institute (b)		
Cinnamyl anthranilate	4/48	4/48
2,6-Toluenediamine · 2 HCl	2/50	2/50
4,4'-Oxydianiline	9/50	9/50
Di(2-ethylhexyl)adipate	16/50	16/50
Di(2-ethylhexyl)phthalate	8/50	8/50
Butyl benzyl phthalate	13/50	14/50
Locust bean gum	12/50	12/50
Gum arabic	9/49	9/49
Guar gum	7/50	7/50
Tara gum	6/50	6/50
Agar	2/49	3/49
2-Biphenylamine · HCl	6/50	6/50
4,4'-Methylenedianiline · 2 HCl	10/49	10/49
Monuron	3/50	3/50
8-Hydroxyquinoline	12/50	12/50
TOTAL	119/745 (16.0%)	121/745 (16.2%)
SD (c)	8.43%	8.42%
Range (d)		
High	16/50	16/50
Low	2/50	2/50
Overall Historical Incidence		
TOTAL	281/2,395 (11.7%)	298/2,395 (12.4%)
SD (c)	6.81%	7.08%
Range (d)		
High	16/50	16/50
Low	1/52	1/52

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Test results are listed chronologically by terminal kill date from cinnamyl anthranilate (December 1976) through 8-hydroxyquinoline (December 1981).
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

TABLE F18. HISTORICAL INCIDENCE OF LUNG TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Alveolar /Bronchiolar Adenoma	Alveolar /Bronchiolar Carcinoma	Alveolar /Bronchiolar Adenoma or Carcinoma
Historical Incidence at Mason Research Institute			
4,4'-Methylenedianiline · 2HCl	1/50	1/50	2/50
Monuron	4/50	2/50	6/50
8-Hydroxyquinoline	1/49	1/49	2/49
Butyl benzyl phthalate	5/50	3/50	8/50
Di(2-ethylhexyl)phthalate	0/50	0/50	0/50
Di(2-ethylhexyl)adipate	5/49	1/49	6/49
Guar gum	2/50	3/50	5/50
Locust bean gum	2/50	3/50	5/50
Gum arabic	2/48	1/48	3/48
Tara gum	7/50	1/50	8/50
Agar	5/50	2/50	7/50
2,6-Toluenediamine · 2HCl	4/50	0/50	4/50
4,4'-Oxydianiline	5/50	0/50	5/50
2-Biphenylamine · HCl	6/49	0/49	6/49
Cinnamyl anthranilate	3/50	3/50	6/50
TOTAL	52/745 (7.0%)	21/745 (2.8%)	73/745 (9.8%)
SD (b)	4.15%	2.36%	4.59%
Range (c)			
High	7/50	3/50	8/50
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	131/2,439 (5.4%)	49/2,439 (2.0%)	179/2,439 (7.3%)
SD (b)	3.69%	2.33%	4.22%
Range (c)			
High	7/50	4/48	8/50
Low	0/51	0/50	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE F19. HISTORICAL INCIDENCE OF ADRENAL GLAND TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence of Pheochromocytomas in Controls
Historical Incidence at Mason Research Institute	
4,4'-Methylenedianiline·2HCl	1/50
8-Hydroxyquinoline	1/49
Guar gum	1/47
Locust bean gum	1/45
Tara gum	1/46
All others	0/467
TOTAL	5/704 (0.7%)
SD (b)	1.03%
Range (c)	
High	1/45
Low	0/50
Overall Historical Incidence	
TOTAL	(d) 15/2,357 (0.6%)
SD (b)	1.16%
Range (c)	
High	2/50
Low	0/51

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) No malignant pheochromocytomas were observed.

TABLE F20. HISTORICAL INCIDENCE OF HARDERIAN GLAND TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	*Incidence of Adenoma or Adenocarcinoma* in Controls
Historical Incidence at Mason Research Institute	
4,4'-Methylenedianiline · 2HCl	1/50
Monuron	1/50
8-Hydroxyquinoline	1/50
Butyl benzyl phthalate	1/50
Di(2-ethylhexyl)phthalate	0/50
Di(2-ethylhexyl)adipate	1/50
Guar gum	1/50
Locust bean gum	1/50
Gum arabic	2/49
Tara gum	1/50
Agar	1/50
2,6-Toluenediamine · 2HCl	0/50
4,4'-Oxydianiline	2/50
2-Biphenylamine · HCl	0/49
Cinnamyl anthranilate	1/50
TOTAL	14/748 (1.9%)
SD (b)	1.20%
Range (c)	
High	2/49
Low	0/50
Overall Historical Incidence	
TOTAL	(d) 33/2,537 (1.3%)
SD (b)	1.72%
Range (c)	
High	3/48
Low	0/89

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) One papillary cystadenocarcinoma, NOS, was observed; all other diagnoses were adenoma, NOS, papillary adenoma, or cystadenoma, NOS.

TABLE F21. HISTORICAL INCIDENCE OF HEMATOPOIETIC SYSTEM TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls	
	Malignant Lymphoma	Lymphoma or Leukemia
Historical Incidence at Mason Research Institute		
4,4'-Methylenedianiline·2HCl	13/50	13/50
Monuron	16/50	16/50
8-Hydroxyquinoline	13/50	13/50
Butyl benzyl phthalate	17/50	17/50
Di(2-ethylhexyl)phthalate	10/50	10/50
Di(2-ethylhexyl)adipate	23/50	23/50
Guar gum	19/50	19/50
Locust bean gum	31/50	31/50
Gum arabic	18/49	19/49
Agar	9/50	9/50
Tara gum	16/50	16/50
2,6-Toluenediamine·2 HCl	4/50	4/50
4,4'-Oxydianiline	15/50	15/50
Cinnamyl anthranilate	18/50	18/50
2-Biphenylamine·HCl	10/49	10/49
TOTAL	232/748 (31%)	233/748 (31.1%)
SD (b)	12.78%	12.85%
Range (c)		
High	31/50	31/50
Low	4/50	4/50
Overall Historical Incidence		
TOTAL	637/2,537 (25.1%)	689/2,537 (27.2%)
SD (b)	10.03%	9.87%
Range (c)		
High	31/50	31/50
Low	4/50	4/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE F22. HISTORICAL INCIDENCE OF LIVER TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Mason Research Institute			
4,4'-Methylenedianiline · 2HCl	3/50	1/50	4/50
Monuron	5/50	2/50	6/50
8-Hydroxyquinoline	2/49	3/49	5/49
Butyl benzyl phthalate	0/50	2/50	2/50
Di(2-ethylhexyl)phthalate	1/50	0/50	1/50
Di(2-ethylhexyl)adipate	2/50	1/50	3/50
Guar gum	2/50	4/50	5/50
Locust bean gum	1/49	2/49	3/49
Gum arabic	2/49	1/49	3/49
Agar	1/50	3/50	4/50
Tara gum	9/49	1/49	10/49
2,6-Toluenediamine · 2 HCl	4/50	0/50	4/50
4,4'-Oxydianiline	4/50	4/50	8/50
2-Biphenylamine · HCl	3/49	4/49	7/49
Cinnamyl anthranilate	2/50	1/50	3/50
TOTAL	41/745 (5.5%)	29/745 (3.9%)	68/745 (9.1%)
SD (b)	4.44%	2.79%	4.85%
Range (c)			
High	9/49	4/49	10/49
Low	0/50	0/50	1/50
Overall Historical Incidence at all Laboratories			
TOTAL	102/2,519 (4%)	106/2,519 (4.2%)	205/2,519 (8.1%)
SD (b)	3.9%	3.09%	4.75%
Range (c)			
High	9/49	7/48	10/49
Low	0/50	0/50	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

APPENDIX G

GENETIC TOXICOLOGY OF

C.I. BASIC RED 9 MONOHYDROCHLORIDE

TABLE G1. MUTAGENICITY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE IN *SALMONELLA TYPHIMURIUM*

Strain	Dose (µg/plate)	Revertants/plate (a,b) −S9	+S9 (rat)	+S9 (hamster)
TA100	0	98 ± 1.8	96 ± 6.4	136 ± 10.7
	1	107 ± 4.5	--	--
	3	95 ± 3.4	--	--
	10	84 ± 2.2	112 ± 7.8	180 ± 16.8
	33	114 ± 5.3	134 ± 4.8	253 ± 13.0
	100	42 ± 14.3	152 ± 10.7	349 ± 44.6
	333	--	180 ± 7.2	574 ± 24.4
	666	--	--	579 ± 5.2
	1,000	--	143 ± 13.2	--
TA1535	0	22 ± 2.7	6 ± 0.7	6 ± 0.3
	1	27 ± 4.8	--	--
	3	29 ± 3.7	--	--
	10	32 ± 2.4	10 ± 1.8	10 ± 1.7
	33	25 ± 0.3	9 ± 3.4	6 ± 1.2
	100	7 ± 3.5	7 ± 1.0	9 ± 1.8
	333	--	8 ± 2.1	6 ± 0.9
	1,000	--	12 ± 2.0	11 ± 1.5
TA1537	0	7 ± 0.7	6 ± 0.9	7 ± 0.6
	1	8 ± 3.7	--	--
	3	5 ± 0.9	--	--
	10	7 ± 0.7	7 ± 1.0	7 ± 1.9
	33	5 ± 0.3	9 ± 2.3	9 ± 1.5
	100	5 ± 1.0	7 ± 1.2	12 ± 0.3
	333	--	10 ± 1.3	8 ± 2.5
	1,000	--	9 ± 2.3	10 ± 2.8
TA98	0	16 ± 1.2	20 ± 2.8	19 ± 0.3
	1	10 ± 2.0	--	--
	3	17 ± 1.2	--	--
	10	17 ± 0.9	31 ± 3.1	28 ± 2.3
	33	14 ± 0.9	38 ± 0.7	36 ± 4.5
	100	17 ± 2.1	28 ± 4.6	48 ± 6.2
	333	--	41 ± 6.1	76 ± 7.0
	666	--	--	64 ± 10.9
	1,000	--	32 ± 0.9	--

(a) The S9 fractions were prepared from the livers of Aroclor 1254-induced male Sprague-Dawley rats and male Syrian hamsters. Cells and test compound or solvent (DMSO) were incubated for 20 min at 37° C in the presence of either S9 or buffer. After the addition of soft agar, the contents of each tube were poured onto minimal medium, and the plates were incubated at 37° C for 48 h (Haworth et al., 1983). The experiment was performed twice, each in triplicate; because the results were similar, data from only one experiment are shown.
(b) Mean ± standard error

TABLE G2. MUTAGENICITY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE IN L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS IN THE PRESENCE OF S9 (a)

Compound	Dose (μg/ml)	Total Mutant Clones	Cloning Efficiency (percent)	Relative Total Growth (percent)	Mutation Frequency (mutants/10^6 clonable cells)
DMSO					
	(1%)	156	95.0	100	55
		105	87.2	100	40
		93	77.7	100	40
		106	55.5	100	64
3-Methylcholanthrene					
	5	412	38.0	2.3	361
		403	57.3	4.4	234
		338	38.2	3.0	295
C.I. Basic Red 9 monohydrochloride					
	33	154	75.3	53.6	68
		99	144.0	95.0	23
	41	253	68.8	41.4	123
		243	87.0	53.7	93
	51	284	52.5	26.2	180
		455	64.2	30.4	236
	64	278	47.2	18.5	196
		163	24.0	9.1	226
	80	56	13.5	3.7	138
		552	18.2	5.6	1,013
	100	123	1.7	0.3	2,460
		41	1.0	0.2	1,367

(a) Experiments were performed twice; all doses were tested in duplicate, except the solvent control (water), which was tested in triplicate. Because the results were similar, data from only one experiment are shown. The protocol was basically that of Clive et al. (1979). Cells (6×10^5/ml) were treated for 4 h at 37° C in medium, washed, resuspended in medium, and incubated for 48 h at 37° C. After expression, 3×10^6 cells were plated in medium supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium to determine the percentage of viable cells. S9 was prepared from the livers of Aroclor 1254-induced male F344 rats.

TABLE G3. MUTAGENICITY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE IN L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS IN THE ABSENCE OF S9 (a)

Compound	Dose (μg/ml)	Total Mutant Clones	Cloning Efficiency (percent)	Relative Total Growth (percent)	Mutation Frequency (mutants/10^6 clonable cells)
DMSO					
	(1%)	48	81.0	100	20
		62	85.2	100	24
Ethylmethanesulfonate					
	500	444	47.8	5.8	309
		424	52.2	6.3	271
C.I. Basic Red 9 monohydrochloride					
	0.512	107	103.5	105.2	34
		131	124.2	120.6	35
	1.02	112	98.5	81.0	38
		146	61.3	48.5	79
	2.05	94	82.5	48.8	38
		100	74.3	43.0	45
	4.10	67	20.3	7.4	110
		72	26.7	9.8	90
	5.12	60	11.5	3.3	174
		17	9.8	3.2	58

(a) Experiments were performed twice, and all doses were tested in duplicate, except the solvent control (DMSO), which was tested in triplicate. Because the results were similar, data from only one experiment are shown. The protocol was basically that of Clive et al. (1979). Cells (6×10^5/ml) were treated for 4 h at 37° C in medium, washed, resuspended in medium, and incubated for 48 h at 37° C . After expression, 3×10^6 cells were plated in medium supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium to determine the percentage of viable cells.

TABLE G4. INDUCTION OF SISTER-CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY C.I. BASIC RED 9 MONOHYDROCHLORIDE

−S9 (b)		+S9 (c)	
Dose (µg/ml)	SCE/Cell	Dose (µg/ml)	SCE/Cell
DMSO (10 µl)	10.6	DMSO (10 µl)	9.6
C.I. Basic Red 9 monohydrochloride		C.I. Basic Red 9 monohydrochloride	
2.5	10.7	20	11.4
5.0	11.7	25	12.6
7.5	12.0	35	13.3
Mitomycin C		Cyclophosphamide	
0.01	47.5	2.0	47.4
0.001	13.2	0.4	16.8

(a) SCE, sister-chromatid exchange; CHO, Cinese hamster ovary
(b) In the absence of S9, CHO cells were incubated with test compound or solvent for 2 h at 37° C. Then BrdU was added, and incubation continued for 24 h. Cells were washed, fresh medium containing BrdU (10 µM) and colcemid (0.1 µg/ml) was added, and incubation was continued for 2-3 h. Cells were then collected by mitotic shake-off, treated for 3 min with KCl (75 mM), washed twice with fixative, and dropped onto slides and air dried. Staining was by a modified technique (after Perry and Wolff, 1974; Goto et al., 1978).
(c) In the presence of S9, cells were incubated with test compound or solvent for 2 h at 37° C. Then cells were washed, and medium containing 10 µM BrdU was added. Cells were incubated for a further 26 h, with colcemid (0.1 µg/ml) present for the final 2-3 h. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

TABLE G5. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY C.I. BASIC RED 9 MONOHYDROCHLORIDE

−S9 (b)		+S9 (c)	
Dose (µg/ml)	Abs/100 Cells (percent cells w/abs)	Dose (µg/ml)	Abs/100 Cells (percent cells w/abs)
DMSO (10 µl)	>4 (4)	DMSO (10 µl)	4 (3)
C.I. Basic Red 9 monohydrochloride		C.I. Basic Red 9 monohydrochloride	
10.0	>6 (5)	40	>3 (3)
12.5	>5 (6.3)	45	1 (1)
15.0	>5 (4)	50	>6 (5)
Mitomycin C		Cyclophosphamide	
0.08	72 (56)	17.5	38 (32)

(a) Abs, aberration; CHO, Cinese hamster ovary
(b) In the absence of S9, CHO cells were incubated with test compound or solvent for 8-10 h at 37° C. Cells were then washed, and fresh medium containing colcemid (0.1 µg/ml) was added. After a further 2-3 h of incubation, cells were harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.
(c) In the presence of S9, cells were incubated with test compound or solvent for 2 h at 37° C. Cells were then washed, medium was added, and incubation continued for 8-10 h. Colcemid (0.1 µg/ml) was added for the last 2-3 hours of incubation; then cells were harvested and fixed as described in footnote (a). S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

TABLE G6. INDUCTION OF UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES BY C.I. BASIC RED 9 MONOHYDROCHLORIDE

Compound (a)	Dose (µg/ml)	Net Grains per Nucleus ± Standard Error
DMSO	1%	−13.9 ± 3.4
2-Acetylaminofluorene	5.63	59.9 ± 5.6
C.I. Basic Red 9 monohydrochloride	0.018	−10.7 ± 4.1
	0.088	−6.8 ± 1.4
	0.44	−8.0 ± 0.4
	2.20	7.8 ± 6.0

(a) Unscheduled DNA synthesis was determined essentially by the method of Williams (1977). Hepatocytes from male F344 rats were isolated according to the procedure of Williams et al. (1977); inoculated into Williams Medium E supplemented with 2 mM glutamine, 50 µg/ml gentamicin, and 10% fetal bovine serum; and allowed to attach for 2 h. After incubation, the cells were washed, and serum-free medium was added. Three cultures were used per dose of compound (and for controls), and cultures were exposed simulataneously to the test compound and to tritiated thymidine (10 µCi/ml) for 18 h. After exposure, cultures were washed, swelled in a hypotonic solution, fixed, and washed with water. The coverslips were mounted to slides, dipped in Kodak NTB-2 emulsion, and exposed at 20° C for 6 d. Cells were stained with methyl-green Pyronin. The grains over 50 morphologically unaltered cells were counted, and the highest count from two nuclear-sized areas over the most heavily labeled cytoplasmic areas adjacent to the nucleus were subtracted from the nuclear count to obtain the net grains per nucleus.

APPENDIX H

CHEMICAL CHARACTERIZATION OF

C.I. BASIC RED 9 MONOHYDROCHLORIDE

APPENDIX H. CHEMICAL CHARACTERIZATION

I. Identity and Purity Determinations Performed by the Analytical Chemistry Laboratory

A. Lot No. PO1340

1. Physical Properties

a. Appearance: Metallic green microcrystals

b. Melting Point:

Determined	Literature Values
Begins decomposition at 220° C with continued changes in appearance to 320° C where charring begins (visual, sealed capillary). Overlapping exotherms; 224°-231° C and 232°-241° C. Overlapping endotherms; 335°-344° C and 365°-372° C (Dupont 900 DTA)	No literature reference found

2. Spectral Data

a. Infrared

	Determined	Literature Values
(1) Instrument:	Beckman IR-12	
(2) Phase:	0.5% Potassium bromide pellet	
(3) Results:	See Figure 5	Consistent with literature spectrum (Sadtler Standard Spectra)

b. Ultraviolet/Visible

	Determined		Literature Values	
(1) Instrument:	Cary 118			
(2) Solvent:	Methanol		Methanol	
(3) Results:	λ_{max} (nm)	$\varepsilon \times 10^{-4}$	λ_{max} (nm)	$\varepsilon \times 10^{-4}$
	547	9.37 ± 0.03 (δ)	263	1.05
	289	1.99 ± 0.02 (δ)	The ultraviolet spectrum obtained at Midwest Research Institute was not consistent with this literature value (Sadtler Standard Spectra) but was consistent with the structure of C.I. Basic Red 9 monohydrochloride.	
	239	1.34 ± 0.01 (δ)		

FIGURE 5. INFRARED ABSORPTION SPECTRUM OF C. I. BASIC RED 9 MONOHYDROCHLORIDE (LOT NO. PO1340)

c. Nuclear Magnetic Resonance

	Determined	Literature Values
(1) Instrument:	Varian EM-360A	
(2) Solvent:	Dimethyl sulfoxide-d_6:D_2O (1:1, v:v) with internal tetramethylsilane	
(3) Assignments:	See Figure 6	No literature reference found. Spectrum is consistent with that expected for structure.
(4) Chemical Shift (δ):	a d, 6.78 ppm b d, 7.10 ppm	
(5) Coupling Constant:	$J_{a\text{-}b}$ 9Hz	
(6) Integration Ratios:	a 6.35 b 5.65	

3. Titration: Nonaqueous titration of three amine functions with perchloric acid (mercuric acetate added to complex the chloride ions)
92.8% ± 1.5 (δ)%

4. Water Analysis (Karl Fischer): 9.1% ± 0.1 (δ)%

5. Elemental Analysis

Element	C	H	N	Cl
Theory	70.47	5.60	12.98	10.95
Theory calculated for 9.1% water	64.06	6.10	11.80	9.95
Theory calculated for 92.8% purity by titration	65.40	6.00	12.05	10.16
Determined	65.80 65.88	6.14 6.05	12.21 12.29	10.28 10.41

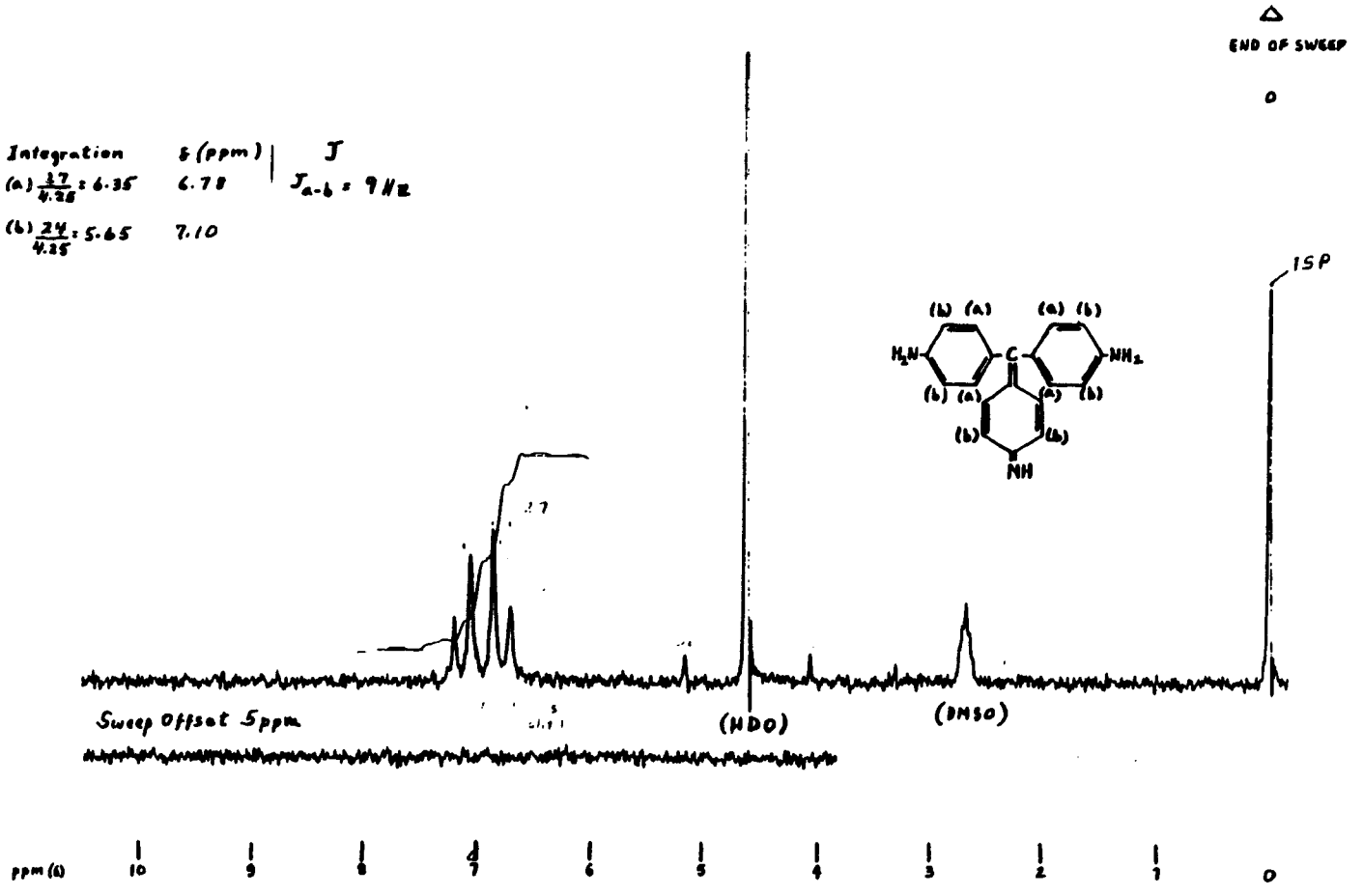

FIGURE 6. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (LOT NO. PO1340)

6. Chromatographic Analysis

a. Thin-Layer Chromatography

(1) Plates: Silica Gel 60 F-254

(2) Reference Standard: *p*-Aminoacetanilide, 10 µg (10 µg/µl in methanol)

(3) Amount Spotted: 100 and 300 µg (10 µg/µl in methanol)

(4) Visualization: Visible and ultraviolet light (254 nm and 366 nm) and furfural spray (1 drop/1 ml glacial acetic acid). Furfural darkens visible spots but detects no additional spots.

System 1: *n*-Butanol:ethanol:water (80:15:5)

	R_f	R_{st}
Trace	Origin	Origin
Trace	0.03	0.05
Trace	0.29	0.47
Slight trace	0.34	0.55
Slight trace	0.37	0.59
Major	0.63	1.02
Trace	0.66	1.07
Trace	0.70	1.14

System 2: Isopropanol: ammonium hydroxide (95:5) (Programmed multiple development: Solvent was allowed to migrate 1 cm then 2 cm, 4 cm, 8 cm, and 16 cm with plate allowed to dry between each successive development.)

	R_f	R_{st}
Minor	Origin	Origin
Trace	0.13	0.20
Trace	0.21	0.34
Trace	0.23	0.35
Trace	0.25	0.38
Trace	0.28	0.43
Trace	0.33	0.50
Major	0.46	0.70
Trace	0.62	0.94
Trace	0.64	0.97
Trace	0.68	1.00
Slight trace	0.73	1.10

b. High-Performance Liquid Chromatography

(1) Instrument: Waters ALC-201
(2) Column: µBondapak C_{18}, 300 mm × 4 mm ID
(3) Detector: Ultraviolet (254 nm)
(4) Solvent: A--5mM 1-Heptane sulfonic acid sodium salt in 1% aqueous acetic acid (v:v); B--5mM 1-Heptane sulfonic acid sodium salt in 1% methanolic acetic acid (v:v)
(5) Solvent Program: 75 min at 55% B, then 55%-100% B in 15 min (linear program)
(6) Solvent Flow Rate: 1 ml/min
(7) Sample Injected: 25 µl containing 1 mg/ml of C.I. Basic Red 9 monohydrochloride in methanol

(8) Results: Major peak and no impurities. The major peak had a retention time of 10.8 minutes.
(See section B.6.b. of this appendix for additional high-performance liquid chromatography analytical information on lot no. PO1340.)

B. Lot No. A7X

1. Appearance: Dark crystalline powder with green luster

2. Spectral Data

a. Infrared

	Determined	Literature Values
(1) Instrument:	Beckman IR-12	
(2) Phase:	0.2% Potassium bromide pellet	
(3) Results:	See Figure 7	Consistent with literature spectrum (Sadtler Standard Spectra)

b. Ultraviolet/Visible

	Determined	Literature Values
(1) Instrument:	Cary 118	
(2) Solvent:	Methanol	Methanol

(3) Results:

Determined λ_{max} (nm)	Determined $\varepsilon \times 10^{-4}$	Literature Values λ_{max} (nm)	Literature Values $\varepsilon \times 10^{-4}$
547	10.05 ± 0.07 (δ)	263	1.05
500 (shoulder)	5.64 ± 0.06		
289	2.09 ± 0.01		
239	1.42 ± 0.09		

The ultraviolet spectrum obtained at Midwest Research Institute was not consistent with this literature value (Sadtler Standard Spectra) but was consistent with the structure of C.I.Basic Red 9 monohydrochloride.

c. Nuclear Magnetic Resonance

	Determined	Literature Values
(1) Instrument:	Varian EM-360A	
(2) Solvent:	Deuterated methanol with internal tetramethylsilane	
(3) Assignments:	See Figure 8	Spectrum consistent with structure
(4) Chemical Shift (δ):	a d, 6.83 ppm b d, 7.23 ppm	
(5) Coupling Constant:	$J_{a\text{-}b}$ 9Hz	
(6) Integration Ratios:	a 6.15 b 5.85	

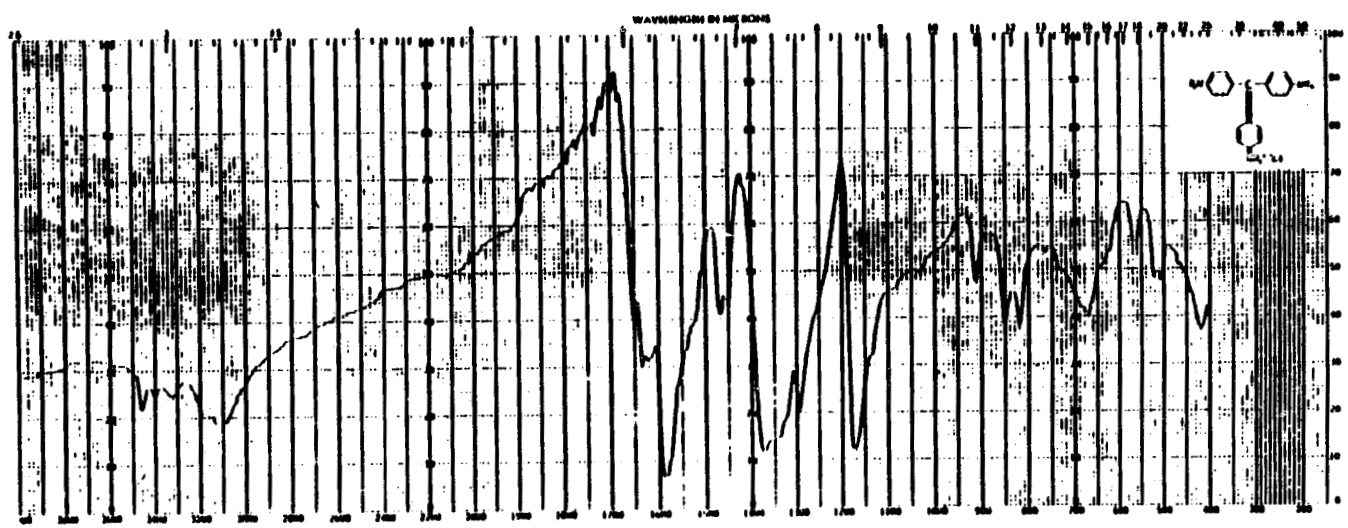

FIGURE 7. INFRARED ABSORPTION SPECTRUM OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (LOT NO. A7X)

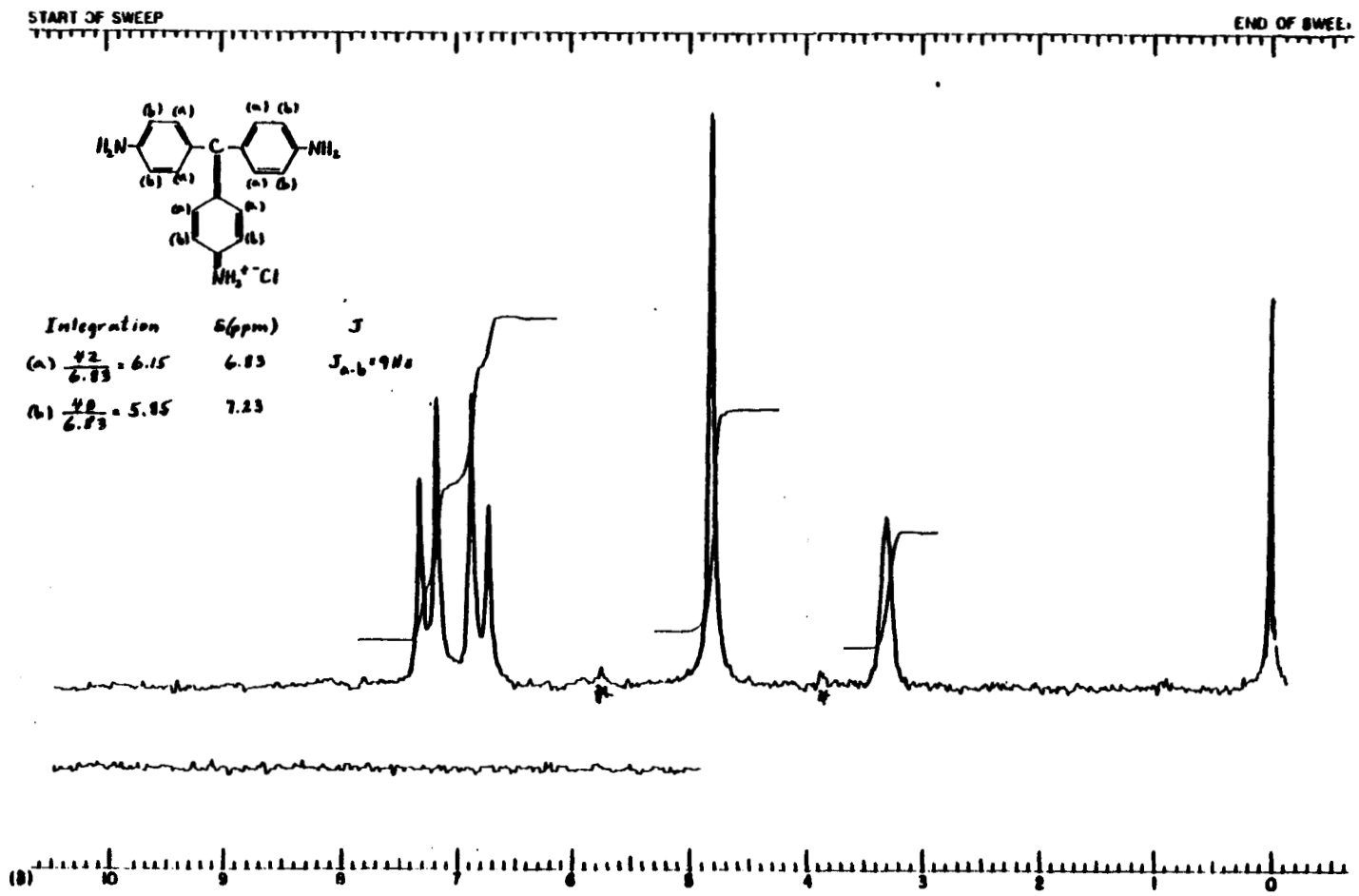

FIGURE 8. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF C. I. BASIC RED 9 MONOHYDROCHLORIDE (LOT NO. A7X)

3. Titration: Nonaqueous titration of one amine group with perchloric acid. The sample was dissolved in glacial acetic acid:acetonitrile (35:10) and mercuric acetate was added to complex the chloride ions.

99.3% ± 0.8 (δ)%

4. Water Analysis (Karl Fischer): 0.55% ± 0.07 (δ)%

5. Elemental Analysis

Element	C	H	N	Cl	S
Theory	70.47	5.60	12.98	10.95	0.00
Determined	70.34	5.67	12.79	10.62	0.10
	70.30	5.79	12.86	10.47	0.11

6. Chromatographic Analysis

a. Thin-Layer Chromatography

(1) Plates: Silica Gel 60 F-254

(2) Reference Standard: *p*-Aminoacetanilide, 10 µg (10 µg/µl in methanol)

(3) Amount Spotted: 100 and 300 µg (10 µg/µl in methanol)

(4) Visualization: Visible and ultraviolet light (254 nm and 366 nm) and furfural spray (1 drop/1 ml glacial acetic acid). Furfural darkens visible spots but detects no additional spots.

	R_f	R_{st}
System 1: *n*-Butanol:ethanol (95%):water (80:15:5)		
Multiple unresolved traces	Origin	Origin
Trace	0.32	0.50
Slight trace	0.39	0.61
Slight trace	0.41	0.64
Trace	0.43	0.67
Major	0.60	0.92
Trace	0.71	1.10
Slight trace	0.90	1.39
Trace	0.95	1.48
System 2: Isopropanol:ammonium hydroxide (95:5)		
Trace	Origin	Origin
Trace	0.11	0.14
Trace	0.28	0.36
Major	0.46	0.61
Trace	0.65	0.85
Trace	0.70	0.92
Slight trace	0.80	1.06
Trace	0.88	1.16
Trace	0.94	1.23

b. High-Performance Liquid Chromatography:

(1) Instrument System:
(a) Pump(s): Waters 6000A
(b) Programmer: Waters 660
(c) Detector: Waters 440
(d) Injector: Waters U6K

(2) Column: μBondapak C_{18}, 300 mm × 3.9 mm ID
(3) Detection: Ultraviolet (254 nm)
(4) Guard Column: CO:PELL ODS, 72 mm × 2.3 mm ID

(5) Solvent System:
A Water with 5mM heptane sulfonic acid sodium salt and 1% (v:v) acetic acid;
B Methanol with 5mM heptane sulfonic acid sodium salt and 1% (v:v) acetic acid

(6) Program:
System 1: 51% A:49% B, isocratic
System 2: 33% A:67% B, isocratic

(7) Flow Rate: 1 ml/min
(8) Samples Injected: Solutions (5 μl for System 1, 15 μl for System 2) of 0.2% C.I. Basic Red 9 monohydrochloride, in methanol, filtered

(9) Results: System 1: Major peak and eight impurities, four before and four after the major peak, totaling 0.83% of the major peak;
System 2: Major peak and seven impurities, one before and six after the major peak, totaling 1.4% of the major peak.

Peak No.	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
System 1			
1	5.2	0.27	0.11
2	12.0	0.61	0.04
3	14.8	0.75	0.02
4	17.0	0.86	0.06
5	19.8	1.00	100
6	25.5	1.29	0.02
7	32.0	1.62	0.42
8	39.5	2.00	0.13
9	44.0	2.23	0.03
System 2			
1	4.2	0.81	0.04
2	5.2	1.00	100
3	8.5	1.62	0.06
4	11.0	2.10	(a) 0.19
5	12.5	2.38	(a) 0.40
6	14.0	2.67	(a) 0.16
7	16.2	3.10	(a) 0.08
8	65.0	12.38	0.46

(a) Peaks 4 through 7 are superimposed on what is probably a number of unresolved impurities rather than tailing of the major peak, judging from the shape of the on-scale major peak.

Lot no. A7X and lot no. PO1340 were compared by using both of the systems above. The two lots were identical by System 1. They were very similar by System 2, the main differences being that in lot no. PO1340 peak number 6 was five times larger (1.3% of major peak) than in lot no. A7X and peak 8 was absent.

II. Test Chemical Stability Study Performed by the Analytical Chemistry Laboratory

Bulk Chemical Heat Stability

1. Storage and Analysis: Samples of the compound were stored at −20°, 5°, 25°, and 60° C for 2 weeks. Samples were then analyzed by high-performance liquid chromatography. The system used was the same as for the analytical system in Section I. A. 6. b., except that the system was run isocratically at 55% B with no solvent program. The sample stored at −20° C was used as a standard against which all other samples were compared.

Samples Injected: Solutions (25 µl) were injected containing the samples at a concentration of 1 mg/ml in methanol

2. Results: Retention time of major peak was 10 minutes.

Storage Temperature (degrees centigrade)	Areas (relative to −20° C standard)
−20	100% ± 3.5 (δ)%
5	93.4% ± 1.2 (δ)%
25	104.2% ± 4.8 (δ)%
60	103.9% ± 1.3 (δ)%

3. Conclusion: The chemical is stable for 2 weeks at temperatures up to 60° C.

III. Test Chemical Stability at the Testing Laboratory

Periodic comparisons were made between the bulk chemical and a reference sample stored at −18° C to verify the integrity of the test material.

A. Analytical Methods

1. **Identity Determination:** Infrared spectroscopy
   Instrument: Perkin-Elmer Infracord #137
   Phase: Potassium bromide pellet

2. **Purity Determination:** Thin-layer chromatography
   Plates: Silica gel (Quantum LQDF or Whatman LD5DF)
   Solvent System: *n*-Butanol:ethanol:water (80:15:5)
   Amount Spotted: 10 µg in 10 µl methanol

B. Results:

1. **Identity:** All bulk and reference spectra were essentially identical to each other and to the spectra supplied by the analytical chemistry laboratory.

2. **Purity:**

Date of Analysis	Lot No.	R_f of Major Spot	R_f of Impurities
3/22/78	PO1340	0.52	(a) 0.2 (trace)
7/14/78		0.53	(a) 0.19 (trace)
11/27/78		0.39	0.48 (trace)
3/26/79		0.38	0.48 (trace)
8/06/79		0.68	0.44, 0.53 (trace)
8/06/79	A7X	0.68	0.44, 0.53 (trace)
11/12/79 (b)		0.64	0.41, 0.51 (trace)
4/01/80		0.64	0.41, 0.51 (trace)
7/17/80 (c)		0.65	0.40, 0.44 (trace)
11/13/80		0.68	0.34, 0.45 (trace)
3/20/81		0.67	0.41, 0.44 (trace)
8/01/81		0.64	0.38, 0.45 (trace)

(a) Solvent ratio: 80:15:4.5
(b) 10 µg spotted for this and subsequent analyses; 10 µg in 10 µl methanol spotted for previous analyses
(c) Whatman LD5DF plates were used for this and subsequent analyses. Quantum LQDF was used for previous analyses.

APPENDIX I

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

APPENDIX I. PREPARATION AND CHARACTERIZATION

I. Two-Week Stability Study on Formulated Diets at a Concentration of 2,000 ppm at Four Different Temperatures Conducted at the Analytical Chemistry Laboratory

A. Sample Preparation and Storage: A stock solution of C.I. Basic Red 9 monohydrochloride was prepared in absolute ethanol at a concentration of 1.010 mg/ml. This solution was used to prepare the spiked feed samples and the analysis standard. Each 5-g feed sample for spiking was mixed with 10 ml of the stock solution, and the solvent ethanol was removed on a rotary evaporator (bath temperature, 35° C). Stability test samples were prepared, in duplicate, in exactly the same way and then stored for 2 weeks at −20°, 5°, 25°, or 45° C. The analysis standard was prepared by tenfold dilution of the stock solution (resulting concentration, 0.1010 mg/ml).

B. Extraction and Analysis: Each 5-g feed sample was transferred to a 200-ml centrifuge bottle with two 25-ml portions of methanol. This mixture was vigorously agitated with a Brinkmann Polytron® high-speed blender for 30 seconds and then placed in an ultrasonic vibratory bath for 1 minute. After a 15-minute centrifugation, the supernatant solution was decanted into a 100-ml volumetric flask. The feed residue was reextracted in the same manner with another 50 ml of methanol, and the two supernatant solutions were combined. The final volume was adjusted to 100 ml with methanol. This final solution was filtered through a 0.5-μ Millipore FH filter and analyzed by the high-performance liquid chromatographic system described in Appendix H, section A. 6. b., except that the system was operated isocratically at 55% B.

C. Results

Storage Temperature	Average Percent Chemical Found in Chemical/Vehicle Mixture (a,b)
−20° C	0.211 ± 0.01
5° C	0.206 ± 0.06
25° C	0.181 ± 0.01
45° C	0.166 ± 0.06

(a) Corrected for a spiked recovery yield of 90% ± 2%.
(b) Original concentration, 0.2016% ± 0.0001%. (This corresponds to a C.I. Basic Red 9 monohydrochloride dose of 0.1789%.)

D. Conclusions: C.I. Basic Red 9 monohydrochloride is stable for 2 weeks when mixed with stock rodent feed at the 2,000-ppm concentration and stored at 5° C or below. Samples stored at 25° and 45° C gave determinations that were less than the original concentration. These results might have been due to chemical decomposition or to a physical transformation that rendered the compound less accessible to extraction from the feed.

II. Homogeneity Studies for Mixed Feed Conducted at the Analytical Chemistry Laboratory

A. Mixing Procedure

1. Premix: A solution of 3.0029 ± 0.0002 g of C.I. Basic Red 9 monohydrochloride in 225 ml of 95% ethanol was prepared and added to 200 g of Wayne Lab-Blox® rodent feed in a 1,000-ml round bottom flask. The ethanol was then removed on a rotary evaporator (water aspirator; heating bath, 30° C).

2. Bulk Mixing: The above premix and 1,297 g additional feed were mixed in a Patterson-Kelly Twin-Shell Blender® for a total of 15 minutes. The blender was loaded from the top of the shells as follows: 648 g of feed was poured in and allowed to settle and level at the bottom (vertex of the "V"); the premix was then poured in on top of the feed from each side; this layer was covered with the remaining 649 g of feed poured in from each side. After 10- and 15-minute mixing times, duplicate 5-g samples were removed from the top of each shell and the bottom trap of the blender for subsequent analysis.

B. Extraction and Analysis Procedures

1. Sample Preparation: The chemical/feed samples (5 g) were quantitatively transferred to 200-ml centrifuge bottles with two 25-ml portions of methanol. The feed mixtures were triturated with this solvent with a Brinkmann Polytron® high-speed blender, placed in an ultrasonic vibratory bath for 2 minutes, and then centrifuged for 5 minutes. The supernatant solutions were decanted into 100-ml volumetric flasks. The feed residues were each mixed again with a 40-ml portion of methanol and placed in the ultrasonic vibratory bath for 2 minutes. After centrifugation, these methanolic supernatants were combined with the first extracts and brought to volume with additional methanol. An aliquot of each solution (3 ml) was filtered through a 0.5-μ, syringe-mounted, Millipore filter. The filtrates were analyzed by high-performance liquid chromatography.

2. Analysis

a. Quality Control Procedures: A stock standard solution of C.I. Basic Red 9 monohydrochloride was prepared by dissolving 0.2032 ± 0.0001 g of the chemical in 200.0 ± 0.1 ml (volumetric flask) of 95% ethanol, resulting in a concentration of 1.016 ± 0.001 mg/ml. This solution was further diluted with 95% ethanol to provide three standard solutions with concentrations of 0.0305, 0.0610, and 0.1016 mg/ml for the chromatographic analysis and to establish the linearity of the chromatographic system. A least-squares best-fit plot was calculated, which yielded a linear correlation coefficient of 0.9998 for the standard solution data points. Blank feed sample extracts showed that there was no interference to the analysis from the feed matrix.

Duplicate spiked feed mixtures were allowed to stand for 4 hours before being extracted and analyzed. A second duplicate pair of spikes was extracted as soon as they were prepared.

b. Instrumental Parameters

Instrument: Waters Associates Programmable Component Liquid Chromatography System
Column: μ Bondapak C_{18}, 300 mm × 4 mm ID
Detector: Spectrophotometer, 546 nm
Solvent: 5mM heptane sulfonic acid in 1% aqueous acetic acid, 45%; 5mM heptane sulfonic acid in 1% methanolic acetic acid, 55%
Solvent Flow Rate: 1 ml/min
Retention Time: 6.5 min

c. Results: (Average of two independent homogeneity mixtures)

Sample Position	Sampling Time (minutes)	Average Percent C.I. Basic Red 9 Monohydrochloride Found in Chemical/Vehicle Mixture (a, b)
Right 1	10	0.19 ± 0.01
Right 2	10	0.16 ± 0.01
Left 1	10	0.20 ± 0.01
Left 2	10	0.18 ± 0.01
Bottom 1	10	0.19 ± 0.01
Bottom 2	10	0.19 ± 0.01
Right 1	15	0.18 ± 0.01
Right 2	15	0.18 ± 0.01
Left 1	15	0.17 ± 0.01
Left 2	15	0.18 ± 0.01
Bottom 1	15	0.17 ± 0.01
Bottom 2	15	0.18 ± 0.01

(a) Corrected for a 4-hour spiked recovery yield of 90% ± 3%; zero-time spike recovery yield, 98 ± 3%.
(b) Target concentration of chemical in feed, 0.2002% ± 0.0001% (2,002 ppm)

d. Discussion: The discrepancy between the target percent chemical in feed and the tabulated spike-corrected values has been attributed to a physical absorption process. Just as the zero-time spikes and the 4-hour spikes do not show the same recovery, so the 4-hour spikes and the blender-mixed bulk mixture may show different recoveries due to the latter having been more thoroughly and intimately mixed.

The mean and standard deviation of all uncorrected individual sample determinations in these homogeneity mixing studies (39 values) are 82.8% ± 6.0% of target. Total residence time of the chemical in the feed was 6 hours during the premix drying stage and 15 minutes during the bulk mixing, plus 0-2 hours delay time (in freezer) while other samples were being extracted.

3. **Conclusion:** C.I. Basic Red 9 monohydrochloride mixed with stock rodent feed at the 2,000-ppm concentration yields a more homogeneous mixture after being blended for 15 minutes.

III. Preparation of Formulated Diets at the Testing Laboratory

The formulation procedure was essentially the same as that reported by Midwest Research Institute (Section II. A., above) except that ethanol was not used to prepare the premix. The procedure consisted of homogenizing a chemical/feed premix in a mortar and pestle and then layering the premix between the appropriate amount of feed within a Patterson-Kelley® V-blender. After being mixed for 20 minutes, the formulated diets were transferred to double plastic bags and stored in covered plastic buckets at 0° ± 5° C.

APPENDIX J

METHODS OF ANALYSIS OF FORMULATED DIETS

APPENDIX J. METHODS OF ANALYSIS

I. Testing Laboratory

Procedure: Two-gram samples were extracted with 50 ml of methanol. The supernatants were analyzed by high-performance liquid chromatography under the following conditions:

Instrument: Waters ALC 244
Column: μ Bondapak C_{18}, 300 mm × 4 mm ID
Detection: Spectrophotometric, 546 nm
Solvent System: 5mM heptane sulfonic acid in 1% aqueous acetic acid (45%); 5mM heptane sulfonic acid in 1% methanolic acetic acid (55%)
Solvent Flow Rate: 1 ml/min
Retention Time: 6.5 min

II. Midwest Research Institute

A. Preparation of Standard Spiked Feed: Two working standard solutions of C.I. Basic Red 9 monohydrochloride in methanol were prepared independently at concentrations of 2.42 and 1.95 mg/ml. These solutions were further diluted with methanol to concentrations of 1.45, 1.17, 0.97, and 0.49 mg/ml. Aliquots (10 ml) of the six standard solutions were pipetted into individual 200-ml centrifuge bottles containing 5 g of undosed feed to make spiked feed standards bracketing the specified concentration range of the referee sample. One 200-ml centrifuge bottle containing 5 g of undosed feed was treated with 10 ml of methanol for use as a blank. The spiked feeds and the feed blank were sealed and allowed to remain overnight at room temperature before analysis.

B. Preparation of the Referee Sample: Triplicate weights of the dosed referee feed sample (approximately 5 g weighed to the nearest 0.001 g) were transferred to individual 200-ml centrifuge bottles. A 10-ml aliquot of methanol was pipetted on each sample; then the bottles were sealed and allowed to stand overnight at room temperature with the standards and feed blank before analysis.

C. Analysis Procedure: The next day, 90 ml of methanol was pipetted into each blank, standard, and referee sample bottle. The bottles were placed on a Burrell Model 75 Wrist-Action® shaker and shaken for 15 minutes. After the extraction mixtures were centrifuged for 10 minutes, a 3-ml aliquot of the supernatant solution from each sample bottle was diluted to 250 ml with methanol. The solutions were thoroughly mixed by manual shaking; then the C.I. Basic Red 9 monohydrochloride content of the samples was determined by reading the absorbance of the solutions versus methanol at 547 nm on a Cary 118 spectrophotometer in 1-cm cells.

D. Quality Assurance Measures: The dosed referee feed sample was analyzed in triplicate, and the undosed feed sample was analyzed once. Individually spiked portions of undosed feed (six levels) prepared from two independently weighed standards were treated like the dosed referee feed samples for obtaining standard curve data.

Results were computed from the linear regression equation obtained by plotting the net absorbance of each spiked feed sample versus the amount of chemical in the respective spike feed sample. The linearity of the standard curve data was evaluated by the regression equation.

III. Raltech Scientific Services

A. Receipt and Mixing: The sample, blank, and standard were received in sealed, glass septum vials and were stored in a refrigerator until they were sampled. The vials were mixed for 2 minutes on a Vortex mixer before aliquots were taken for analysis.

B. Standard Preparation: Stock standards (5 mg/lml) were prepared by dissolving 500 mg of standard in 100 ml of absolute ethanol. Dilutions were made on the stock standard to obtain working standards. The standards were 0.005, 0.025, 0.050, 0.100, and 0.150 mg/ml diluted in methanol.

C. Sample Preparation and Analysis: The 5-g feed sample aliquots were extracted in a 200-ml centrifuge bottle with 50 ml of methanol with a Brinkmann Polytron® ultrasonic homogenizer for 30 seconds. The bottle was placed in an ultrasonic bath for 1 minute and centrifuged for 15 minutes. The supernatant was decanted into a 100-ml volumetric flask, and the feed residue reextracted with another 50-ml portion of methanol. The combined supernatants were diluted to volume with methanol. An aliquot of the mixed extract was filtered through a 0.5-μ Millipore FH filter and analyzed by high-performance liquid chromatography.

D. Instrumental Parameters

Instrument: Perkin-Elmer Series 3 liquid chromatograph
Column: Waters μ Bondapak C_{18}, 300 mm × 4 mm ID
Detection: Ultraviolet, 254 nm
Solvent System: 5 mM heptane sulfonic acid in 1% aqueous acetic acid (45%); 5 mM heptane sulfonic acid in 1% methanolic acetic acid (55%)
Mode: Isocratic
Sample Injection Volume: 20 μl

E. Quality Assurance: The sample was analyzed in triplicate and the standards and blank, in duplicate. Duplicate recovery samples were prepared by adding 1 ml of the 5 mg/ml stock standard to 5 g of blank feed, mixing, and extracting.

APPENDIX K

RESULTS OF ANALYSIS OF FORMULATED DIETS

TABLE K1. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (a)

Date Mixed	Determined Concentration for Target Concentration of 500 ppm	1,000 ppm	2,000 ppm
6/19/79	520	1,060	1,900
8/21/79	460	950	2,000
11/6/79	510	1,080	1,960
11/27/79	505	1,100	2,000
2/12/80	530	980	2,030
4/15/80	530	1,000	2,200
5/6/80	540	1,100	2,130
8/19/80	520	1,050	2,180
9/9/80	480	1,100	2,080
12/30/80	540	1,080	2,070
3/17/81	490	1,000	2,130
5/19/81	530	1,070	
Mean (ppm)	513	1,048	2,062
Standard deviation	25.1	51.9	93.8
Coefficient of variation (percent)	4.9	5.0	4.5
Range (ppm)	460-540	950-1,100	1,900-2,200
No. of samples	12	12	11

(a) Results of duplicate analysis

TABLE K2. REFEREE SAMPLE DATA IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

Date Mixed	Target Concentration (ppm)	Determined Concentration Testing Laboratory	Analytical Laboratory
8/21/79	500	460	(a) 470
11/27/79	2,000	2,000	1,640
2/12/80	1,000	980	1,000
5/6/80	1,000	1,100	981
12/30/80	1,000	1,080	1,002
5/19/81	500	530	496

(a) This analysis was performed at Raltech Scientific Services; all others were performed at Midwest Research Institute.

APPENDIX L

SENTINEL ANIMAL PROGRAM

APPENDIX L. SENTINEL ANIMAL PROGRAM

I. Methods

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect test results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via viral serology on sera from extra (sentinel) animals in the test rooms. These animals are untreated, and these animals and the test animals are both subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

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

	Hemagglutination Inhibition	Complement Fixation	ELISA
Mice	PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Ectro (infectious ectromelia) Sendai (14 and 24 mo)	M.Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus) Sendai (6 and 12 mo)	MHV (mouse hepatitis virus)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai (12, 18, and 24 mo)	RCV (rat coronavirus) Sendai (6 mo)	

II. Results

Results are presented in Table L1.

TABLE L1. MURINE VIRUS ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE (a)

	Interval (months)	No. of Animals	Positive Serologic Reaction for
RATS			
	6	10/10	RCV
		10/10	Sendai
	12	10/10	PVM
		10/10	RCV
		9/10	Sendai
	18	10/10	PVM
		10/10	RCV
		10/10	Sendai
	24	10/10	PVM
		10/10	RCV
		9/10	Sendai
MICE			
	6	9/14	Sendai
	12	9/15	Sendai
	18	--	None positive
	24	3/10	Sendai

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing and from the control animals just before they were killed; samples were sent to Microbiological Associates, Inc. (Bethesda, MD) for the Animal Disease Screening Program.

APPENDIX M

FEED AND COMPOUND CONSUMPTION BY RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

TABLE M1. FEED AND COMPOUND CONSUMPTION BY MALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control		Low Dose				High Dose			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b) (grams)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b) (grams)	Dose/ Day (c)
4	19.6	229	18.7	229	1.0	82	17.0	219	0.9	155
8	20.0	277	18.3	267	0.9	68	19.9	261	1.0	152
12	18.6	300	17.6	287	0.9	61	16.9	282	0.9	120
16	19.4	341	17.4	335	0.9	52	15.6	322	0.8	97
20	19.3	358	18.1	347	0.9	52	17.6	342	0.9	103
24	19.7	377	18.7	367	0.9	51	18.7	359	0.9	104
28	18.9	394	18.9	380	1.0	50	17.9	373	0.9	96
32	18.3	396	18.1	390	1.0	47	18.4	385	1.0	96
36	19.6	415	18.6	401	0.9	46	17.1	392	0.9	87
40	18.1	426	17.9	412	1.0	43	17.9	400	1.0	89
44	19.6	434	18.3	422	0.9	43	17.9	407	0.9	88
48	20.6	441	19.9	430	1.0	46	19.4	414	0.9	94
52	19.9	446	18.9	434	0.9	43	18.4	416	0.9	89
56	20.1	447	18.1	438	0.9	41	19.1	418	1.0	92
60	19.4	442	19.3	433	1.0	45	17.6	411	0.9	86
64	19.9	448	17.9	439	0.9	41	17.0	415	0.9	82
68	21.3	450	20.9	435	1.0	48	18.3	408	0.9	90
72	19.6	450	18.0	435	0.9	41	18.3	398	0.9	92
76	18.3	454	19.9	437	1.1	45	17.7	409	1.0	87
80	18.4	461	18.6	448	1.0	41	19.3	421	1.0	92
84	18.3	460	18.7	447	1.0	42	29.7	413	1.6	144
88	17.7	462	17.4	449	1.0	39	19.3	403	1.1	96
92	18.0	467	17.6	449	1.0	39	22.9	415	1.3	110
96	19.7	460	18.9	447	1.0	42	24.1	379	1.2	127
100	20.0	453	33.6	439	1.7	76	22.0	396	1.1	111
Mean	19.3	412	19.1	400	1.0	49	19.1	378	1.0	103
SD (d)	0.9		3.1		0.1	11	2.9		0.2	21
CV(e)	4.7		16.2		10.0	22.4	15.2		20.0	20.4

(a) Grams of feed removed from the feeder; not corrected for scatter
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of compound consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE M2. FEED AND COMPOUND CONSUMPTION BY FEMALE RATS IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control		Low Dose				High Dose			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
4	14.0	158	13.7	156	1.0	44	13.9	154	1.0	90
8	13.9	185	12.7	182	0.9	35	11.6	177	0.8	65
12	11.7	196	11.4	190	1.0	30	9.6	180	0.8	53
16	12.6	210	11.7	207	0.9	28	11.4	190	0.9	60
20	14.0	215	14.0	212	1.0	33	12.9	202	0.9	64
24	14.3	221	13.1	216	0.9	30	12.1	206	0.8	59
28	14.3	225	14.3	223	1.0	32	14.3	210	1.0	68
32	15.6	230	14.7	225	0.9	33	13.6	213	0.9	64
36	15.1	234	15.1	231	1.0	33	14.6	219	1.0	67
40	14.7	246	14.6	239	1.0	30	13.9	224	0.9	62
44	14.6	252	13.7	242	0.9	28	12.6	229	0.9	55
48	14.9	257	13.6	251	0.9	27	12.1	235	0.8	52
52	14.4	265	14.3	257	1.0	28	12.3	240	0.9	51
56	14.0	275	13.6	267	1.0	25	13.0	249	0.9	52
60	14.3	282	13.9	270	1.0	26	13.4	250	0.9	54
64	14.6	289	13.6	280	0.9	24	12.9	258	0.9	50
68	15.0	300	14.9	287	1.0	26	13.9	265	0.9	52
72	14.1	306	13.9	295	1.0	23	13.9	271	1.0	51
76	20.3	317	16.1	302	0.8	27	15.4	277	0.8	56
80	15.1	330	15.0	312	1.0	24	15.6	288	1.0	54
84	15.6	336	16.1	320	1.0	25	14.9	294	1.0	51
88	14.6	343	14.3	324	1.0	22	14.1	308	1.0	46
92	14.6	343	14.1	329	1.0	21	13.9	306	1.0	45
96	15.6	348	14.9	332	1.0	22	14.1	314	0.9	45
100	14.6	349	14.9	349	1.0	21	37.1	315	2.5	118
Mean	14.7	268	14.1	260	1.0	28	14.3	243	1.0	59
SD (d)	1.5		1.1		0.1	5	4.9		0.3	15
CV (e)	10.2		7.8		10.0	17.9	34.3		30.0	25.4

(a) Grams of feed removed from the feeder; not corrected for scatter
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of compound consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE M3. FEED AND COMPOUND CONSUMPTION BY MALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control		Low Dose				High Dose			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
4	16.6	30	19.9	29	1.2	342	16.3	30	1.0	543
8	11.9	33	14.0	31	1.2	226	13.1	32	1.1	411
12	14.1	35	12.6	33	0.9	190	12.1	34	0.9	357
16	12.7	37	11.1	34	0.9	164	10.9	35	0.9	310
20	13.6	39	14.0	36	1.0	194	5.3	36	0.4	147
24	16.6	38	14.0	35	0.8	200	12.6	35	0.8	359
28	15.4	39	13.1	37	0.9	178	12.4	37	0.8	336
32	15.4	41	13.4	38	0.9	177	12.9	37	0.8	347
36	16.6	43	14.6	39	0.9	187	14.1	38	0.9	372
40	13.9	44	12.9	40	0.9	161	12.4	40	0.9	311
44	13.1	45	12.6	41	1.0	153	12.1	40	0.9	304
48	16.7	45	14.6	41	0.9	178	14.9	41	0.9	362
52	16.3	46	13.9	42	0.9	165	12.1	41	0.7	296
64	16.9	47	17.4	43	1.0	203	14.1	42	0.8	337
68	14.0	49	13.9	43	1.0	161	13.3	41	0.9	324
72	14.6	47	16.3	42	1.1	194	15.3	40	1.0	382
76	14.7	48	16.4	42	1.1	196	14.6	40	1.0	364
80	16.0	48	14.1	42	0.9	168	14.1	40	0.9	354
84	15.1	47	14.4	41	1.0	176	13.9	39	0.9	355
88	18.1	47	19.4	41	1.1	237	19.4	39	1.1	498
92	15.1	46	15.3	39	1.0	196	16.9	37	1.1	456
96	16.6	45	18.7	39	1.1	240	19.9	37	1.2	537
100	19.4	45	17.6	39	0.9	225	24.0	37	1.2	649
Mean	15.4	43	15.0	39	1.0	196	14.2	38	0.9	379
SD (d)	1.8		2.3		0.1	40	3.6		0.2	103
CV (e)	11.7		15.3		10.0	20.4	25.4		22.2	27.2

(a) Grams of feed removed from the feeder; not corrected for scatter
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of compound consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE M4. FEED AND COMPOUND CONSUMPTION BY FEMALE MICE IN THE TWO-YEAR FEED STUDY OF C.I. BASIC RED 9 MONOHYDROCHLORIDE

	Control		Low Dose				High Dose			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
4	7.7	27	7.1	23	0.9	155	6.4	24	0.8	268
8	6.7	26	6.4	25	1.0	129	6.6	25	1.0	263
12	7.4	28	7.4	27	1.0	138	6.9	27	0.9	254
16	9.3	30	8.4	28	0.9	151	7.3	28	0.8	260
20	8.7	31	10.4	28	1.2	186	10.1	28	1.2	362
24	9.0	33	9.1	31	1.0	147	7.9	31	0.9	253
28	8.1	34	8.4	31	1.0	136	7.9	29	1.0	271
32	7.9	37	7.6	32	1.0	118	6.6	31	0.8	212
36	7.4	39	7.6	33	1.0	115	7.4	32	1.0	232
40	8.1	40	9.1	34	1.1	134	8.6	32	1.1	268
44	6.9	42	7.7	36	1.1	107	7.4	34	1.1	218
48	8.1	44	8.0	36	1.0	111	9.4	34	1.2	277
52	8.1	46	7.6	37	0.9	102	8.1	35	1.0	233
56	8.7	46	8.4	37	1.0	114	8.7	35	1.0	249
60	7.7	48	7.9	38	1.0	103	8.9	35	1.1	253
64	8.0	47	9.4	37	1.2	127	11.1	34	1.4	328
68	8.7	48	9.1	37	1.0	124	12.3	33	1.4	372
72	7.7	49	7.6	36	1.0	105	11.6	33	1.5	351
76	9.4	50	10.6	36	1.1	147	13.4	32	1.4	420
80	7.7	52	11.4	37	1.5	154	16.3	32	2.1	509
84	9.3	53	14.3	37	1.5	193	18.4	32	2.0	576
88	5.0	53	10.6	36	2.1	147	22.7	30	4.5	757
92	9.1	50	15.1	35	1.7	216	29.1	31	3.2	940
96	8.9	51	18.3	35	2.1	261	35.1	31	4.0	1,134
100	10.4	51	20.1	34	1.9	296	28.1	31	2.7	908
Mean	8.2	42	9.9	33	1.2	149	12.7	31	1.6	407
SD (d)	1.1		3.5		0.4	49	8.0		1.0	257
CV (e)	13.4		35.4		33.3	32.9	63.0		62.5	63.1

(a) Grams of feed removed from the feeder; not corrected for scatter
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of compound consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

APPENDIX N

RADIOIMMUNOASSAY OF SERUM THYROXINE

APPENDIX N. SERUM THYROXINE

The SPAC T4 RIA® is a radioimmunoassay kit available from Mallinkrodt, Inc., St. Louis, Missouri, to measure total serum thyroxine (T_4). Anti-T_4 antibodies for this kit are produced in animals (horse, rabbit, sheep, or goat) by parenteral introduction of T_4 coupled to a carrier protein. In the SPAC T4 RIA® assay, the quantity of ^{125}I T_4 bound by a given quantity of antibody is decreased in the presence of unlabeled T_4 and the effect is directly related to the concentration of the unlabeled hormone. Magnesium-8-anilino-1-naphthalene sulfonate is used to inhibit the binding of T_4 to the binding proteins normally present in serum. Standard quantities of T_4 are selected to cover the expected range of T_4 concentrations in the test serum, and a standard curve is prepared by plotting the percent ^{125}I T_4 bound to each T_4 standard versus the respective standard T_4 concentration. Total serum T_4 concentration in the test mixture then is determined by a comparison of the percentage of ^{125}I T_4 bound in test serum sample to the standard curve. A typical standard curve from the C.I. Basic Red 9 monohydrochloride feed studies is shown in Figure 9.

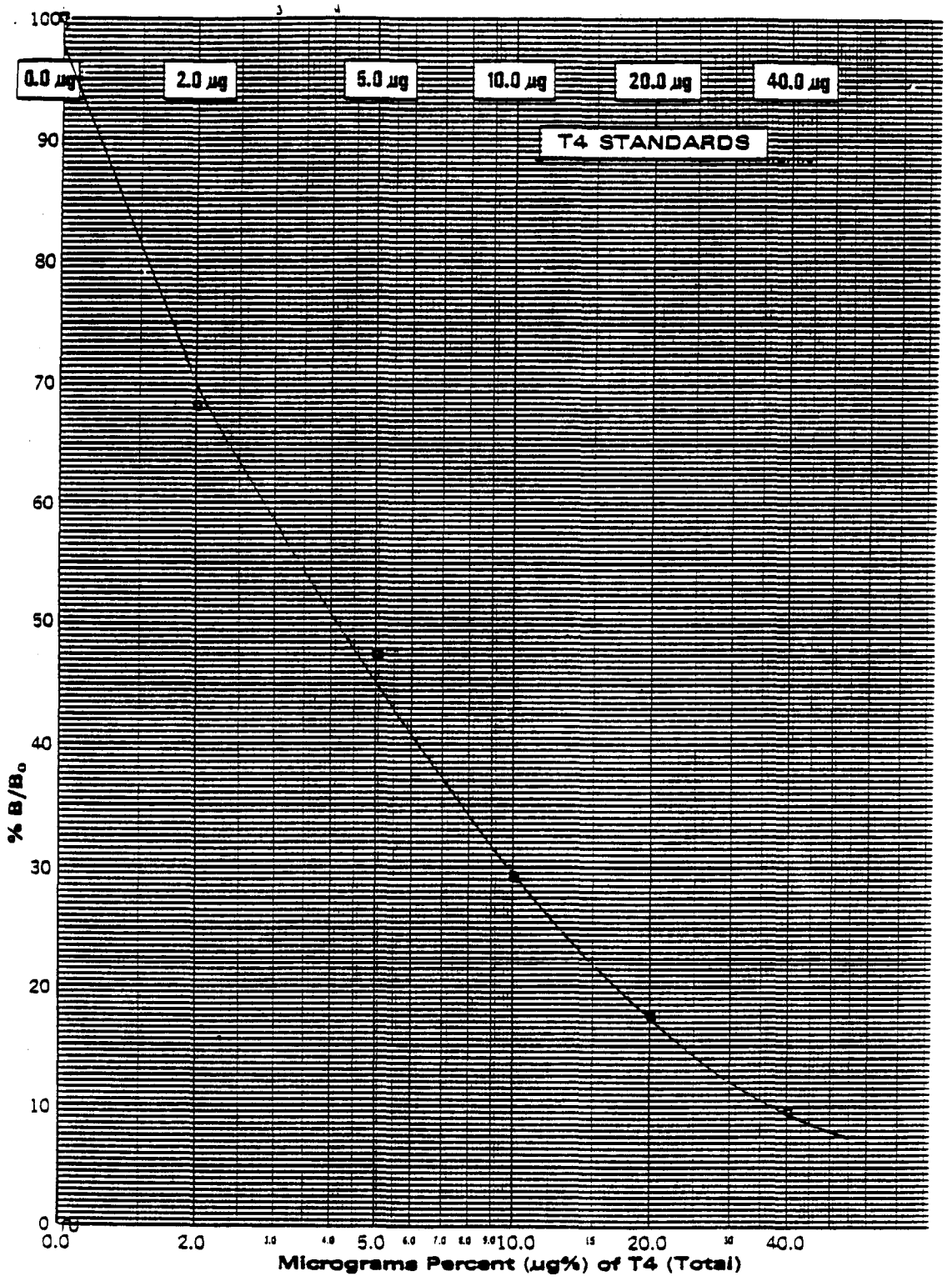

FIGURE 9. TYPICAL STANDARD CURVE FOR RADIOIMMUNOASSAY OF SERUM THYROXINE IN C.I. BASIC RED 9 MONOHYDROCHLORIDE FEED STUDIES

APPENDIX O

DATA AUDIT SUMMARY

APPENDIX O. DATA AUDIT SUMMARY

The toxicology and carcinogenesis feed studies for C.I. Basic Red 9 monohydrochloride in F344/N rats and B6C3F$_1$ mice with a special study to evaluate the effect of the dye on the thyroid gland in F344/N rats were conducted by EG&G Mason Research Institute, Worcester, Massachusetts, under a subcontract with Tracor Jitco .

The audit was conducted on June 25, 1984, by Dr. Jane E. Goeke, Dr. Elizabeth L. Feussner, Dr. Richard E. Long, Mr. Peter D. Ference, Ms. Carol L. Veigle, and Ms. Shirley Cokrsen for Argus Research Laboratories; Mr. Mark Pielmeier and Ms. Gloria Heuckeroth of Tracor Jitco, Inc.; and Ms. Rosalyn N. Joftes of the National Toxicology Program. The audit report is on file at the National Toxicology Program, Research Triangle Park, North Carolina.

The material reviewed in this audit consisted of the following: For the in-life toxicology, 10% of the animal records for C.I. Basic Red 9 monohydrochloride administration, body weight, and clinical observations were audited. All records on animal receipt, acclimation/quarantine, randomization, animal identification, and environmental factors and data for the sentinel and control animals were reviewed. For the analytical chemistry, all records were audited and a random 10% sample of the chemical/vehicle calculations were verified. For pathology, all wet tissue bags were inventoried, and all slides/blocks were matched for the high dose and untreated animals. All individual animal data records, early deaths, and moribund-kill data and data for the target organs and major tissues were audited. Ten percent of the wet tissues were examined, and 10% of the animal identifications were verified.

No data about observations during the acclimation period were available for review, and the basis for randomization at the initiation of the study could not be ascertained. Animals were identified within dose groups by ear punch/notch numbers 1-50. Saving ears with residual wet tissues was not a requirement of the study protocol, and therefore carcass identification could not be confirmed for all animals. No clinical observations were recorded for a few of the animals that were killed in a moribund condition. Clinical observation records did not show the presence of a number of large masses found at necropsy.

The chemical data provided evidence that the level of purity of the C.I. Basic Red 9 monohydrochloride did not change during the course of the study. The chemistry information presented by EG&G Mason Research Institute was supported by the raw data, except that the only bulk chemical analysis data available for auditing were copies of infrared spectrograms and analysis report summaries.

The audit of the pathology materials substantiates the reported findings and conclusions. The principal discrepancies identified concerned mice with multiple hepatocellular tumors. When multiple hepatocellular tumors were present in some mice, only one or two were sectioned and examined by the study pathologist. These discrepancies have no impact on the data interpretation.

In conclusion, the audit substantiates the reported findings and the conclusion of the Technical Report.

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