NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 405

TOXICOLOGY AND CARCINOGENESIS STUDIES OF C.I. ACID RED 114 (CAS NO. 6459-94-5) IN F344/N RATS (DRINKING WATER STUDIES)

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

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF C.I. ACID RED 114

(CAS NO. 6459-94-5)

IN F344/N RATS

(DRINKING WATER STUDIES)

A desalted commercial dye containing approximately 85% 1,3-Naphthalenedisulfonic acid, 8-((3,3′-dimethyl-4′-((4-(((4-methylphenyl)sulfonyl)oxy)phenyl)azo)(1,1′-biphenyl)-4-yl)azo)-7-hydroxy-disodium salt, 10% structurally related compounds, 1%-4% water, and 1% sodium chloride

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

December 1991

NTP TR 405

NIH Publication No. 92-3136

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

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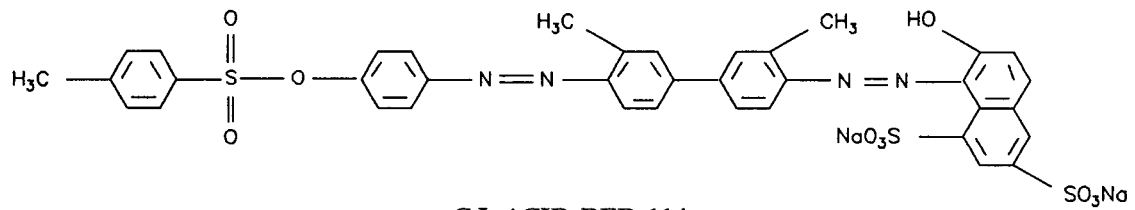
ABSTRACT



C.I. ACID RED 114

CAS No. 6459-94-5

Chemical Formula: $C_{37}H_{28}N_4O_{10}S_3Na_2$ Molecular Weight: 830.8

Synonyms: 1,3-Naphthalenedisulfonic acid, 8-((3,3′-dimethyl-4′-((4-(((4-methylphenyl)sulfonyl)oxy)phenyl)azo)(1,1′-biphenyl)-4-yl)azo)-7-hydroxy, disodium salt, Acid Leather Red BG, Acid Red 114, Amacid Milling Red PRS, Benzyl Fast Red BG, Benzyl Red BR, Cerven Kysela, C.I. 23635, Erionyl Red RS, Folan Red B, Kayanol Milling Red RS, Leather Fast Red B, Levanol Red GG, Midlon Red PRS, Milling Red B, Milling Red BB, Milling Red SWB, NCI C61096, Polar Red RS, Sandolan Red N-RS, Sella Fast Red RS, Sulphonol Fast Red R, Supranol Fast Red GG, Supranol Red PBX-CF, Supranol Red R, Telon Fast Red GG, Tertracid Milling Red B, Vondamol Fast Red RS

C.I. Acid Red 114 is one of five chemicals being evaluated in 2-year carcinogenicity and toxicity studies as part of the NTP's Benzidine Dye Initiative. This Initiative was designed to evaluate representative benzidine congeners, benzidine congener-derived dyes, and benzidine-derived dyes. C.I. Acid Red 114 was nominated for study because of the potential for human exposure during production of bisazobiphenyl dyes and because benzidine, a structurally related chemical, is a known human carcinogen.

Toxicology and carcinogenesis studies were conducted by administering desalted, industrial grade C.I. Acid Red 114 in drinking water to groups of F344/N rats of each sex for 13 days, 13 weeks, 9 or 15 months, or 2 years. These studies were performed only in rats because studies of benzidine congeners were being performed in mice at the National Center for Toxicological Research (NCTR). Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary cells, and *Drosophila melanogaster*.

13-Day Studies

Rats were exposed to C.I. Acid Red 114 in drinking water at doses of 0, 10,000, 20,000, or 30,000 ppm. All control and dosed rats survived except one male rat in the 20,000 ppm dose group. Final mean body weights in the three dosed groups were 94%, 83%, or 77% of controls for males and 92%, 88%, or 80% of controls for females. Water consumption declined with increased dose. Clinical findings included red stained fur, ears, and tail in all test animals. On gross necropsy, organs and tissues were also stained red.

13-Week Studies

C.I. Acid Red 114 was administered in drinking water at doses of 0, 600, 1,200, 2,500, 5,000, or 10,000 ppm. All control and dosed animals survived until the end of the study. Final mean body weights in the five dosed groups were 97%, 89%, 87%, 87%, or 85% of controls for males and 97%, 94%, 94%, 92%, or 89% of controls for females. Water consumption was decreased in dosed animals. As was seen in the 13-day studies, major organs and tissues from treated animals were stained red. Kidney toxicity characterized by regeneration and karyomegaly of tubule epithelial cells with chronic inflammation was observed in female rats at doses of 1,200 ppm or above. Treatment-related increases in relative liver weights and elevated liver enzyme levels were seen in males and females,

centrilobular pallor in the liver was seen in all male dose groups. Because of these body weight differences, decreases in water consumption, and organ toxicity, the doses chosen for the 2-year studies were 70, 150, and 300 ppm for males and 150, 300, and 600 for females.

2-Year Studies

Male rats received doses of 0, 70, 150, or 300 ppm of C.I. Acid Red 114, and female rats received 0, 150, 300, or 600 ppm. Seventy animals were in the control and high-dose groups, 45 in the low-dose groups, and 75 in the mid-dose groups. Ten animals were evaluated from the control and high-dose groups at 9 months, and ten animals from all dose groups were evaluated at 15 months. The average amount of compound consumed per day was 4, 8, or 20 mg/kg for males and 9, 20, or 70 mg/kg for females.

Survival and Body Weights

Survival at 105 weeks for male rats receiving 0, 70, 150, or 300 ppm was 24/50, 15/35, 26/65, and 1/50; for females receiving 0, 150, or 300 ppm, survival was 36/50, 13/35, and 6/64. All female rats receiving 600 ppm died by week 89. The decreased survival in treated groups was due primarily to the development of chemical-related neoplasms. Of the surviving animals, the final mean body weights for males receiving 70 or 150 ppm were 94% and 90% of control and for females receiving 150 or 300 ppm, 99% and 84% of control. These weight differences began in the second year of the studies and were attributed in part to the development of neoplasms in the dosed groups.

Histopathologic Effects in the 2-Year Studies

At 9 and 15 months, a few neoplasms were seen in the liver, lung, clitoral gland, skin, Zymbal's gland, oral cavity epithelium, and small and large intestine, and the number of neoplasms at these sites increased as the studies progressed. At 2 years, there was a clear carcinogenic response in the skin, Zymbal's gland, and liver of male and female rats, and in the clitoral gland, oral cavity epithelium, small and large intestine, and lung in female rats. Treatment-related increases were also seen in the incidence in neoplasms of the oral cavity epithelium, adrenal gland, and lung of male rats, and in mononuclear cell leukemia and in neoplasms of the mammary gland and adrenal gland in female rats. The incidence of these neoplasms was generally lower, but was significant and considered to be marginally related to chemical treatment. The same neoplastic effects have been previously observed in some or all of the NTP studies with dimethoxybenzidine, dimethylbenzidine, or C.I. Direct Blue 15.

Genetic Toxicology

In a standard preincubation protocol, C.I. Acid Red 114 was mutagenic in *Salmonella typhimurium* strain TA98 in the presence of induced hamster liver S9, and an equivocal response was noted in strain TA100 with hamster liver S9. However, no significant mutagenic activity was noted in strains TA1535 or TA1537 with or without S9 activation. In a modified *S. typhimurium* gene mutation test which employed reductive metabolism followed by oxidative metabolism with S9 liver enzymes, C.I. Acid Red 114 was strongly mutagenic in strain TA1538. C.I. Acid Red 114 did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells with or without S9 activation; reductive metabolism was not used in these cytogenetic tests. No increase in sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* administered C.I. Acid Red 114 by feeding or injection.

Conclusions

Under the conditions of these 2-year drinking water studies, there was *clear evidence of carcinogenic activity** of C.I. Acid Red 114 for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, and liver. Increased incidences of neoplasms of the oral cavity epithelium, adrenal gland, and lung may have been related to chemical administration. There was *clear evidence of carcinogenic activity* for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity epithelium, small and large intestines, and lung. Increased incidences of mononuclear cell leukemia, mammary gland adenocarcinoma, and adrenal gland pheochromocytomas may have been related to chemical administration.

*Explanation of Levels of Evidence of Carcinogenic Activity appears on page 8. A summary of peer review comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of C.I. Acid Red 114

Male F344/N Rats	Female F344/N Rats
Drinking water concentration	
0, 70, 150, or 300 ppm C.I. Acid Red 114	0, 150, 300, or 600 ppm C.I. Acid Red 114
Body weights	
Dosed were 9% lower than controls during second year	Dosed were 24% lower than controls during second year
2-Year survival rates[a]	
24/50, 15/35, 26/65, 1/50	36/50, 13/35, 6/64, 0/50
Nonneoplastic effects	
None	None
Neoplastic effects[b]	
Skin basal cell neoplasms: 1/50, 5/35, 28/65, 32/50	Skin basal cell neoplasms: 0/50, 4/35, 7/65, 5/50
Skin keratoacanthoma: 1/50, 1/35, 4/65, 7/50	Zymbal's gland neoplasms: 0/50, 3/35, 18/65, 19/50
Skin sebaceous cell neoplasms: 1/50, 1/35, 5/65, 6/50	Clitoral gland neoplasms: 11/48, 17/32, 28/62, 23/50
Skin squamous cell neoplasms: 1/50, 2/35, 11/65, 9/50	Liver neoplasms: 0/50, 0/35, 19/64, 8/50
Zymbal's gland neoplasms: 0/50, 0/35, 8/65, 7/50	Lung neoplasms: 1/50, 2/35, 9/65, 4/50
Liver neoplasms: 2/50, 2/35, 15/65, 20/50	Oral cavity epithelium neoplasms: 0/50, 3/35, 9/65, 6/50
	Small intestine neoplasms: 0/50, 0/35, 1/65, 2/50
	Large intestine neoplasms: 0/50, 1/35, 0/65, 3/50
Uncertain findings	
Oral cavity epithelium neoplasms: 0/50, 0/35, 1/65, 2/50	Mammary gland adenocarcinoma: 0/50, 3/35, 6/65, 3/50
Adrenal gland pheochromocytomas: 17/50, 11/35, 27/63, 21/49	Adrenal gland pheochromocytomas: 1/50, 3/35, 5/64, 1/50
Lung neoplasms: 2/50, 2/35, 2/65, 3/50	Mononuclear cell leukemia: 12/50, 13/35, 18/65, 5/30
Level of evidence of carcinogenic activity	
Clear evidence	Clear evidence
Genetic toxicology	
Salmonella typhimurium gene mutation:	Positive with S9 in strain TA98; equivocal with S9 in strain TA100; Negative with or without S9 in strain TA1535 or TA1537
Salmonella typhimurium with reductive metabolism:	Positive in strain TA1538
Sister chromatid exchange in Chinese hamster ovary cells *in vitro*:	Negative with or without S9
Chromosomal aberration in Chinese hamster ovary cells *in vitro*:	Negative with or without S9
Drosophila melanogaster germ cell mutation:	Negative by feeding or injection

[a] Reduced survival in exposed groups was due to neoplasia.
[b] Number with lesion/total evaluated

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence)** and **(some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that because of major flaws cannot be evaluated **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.

- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.

- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.

- **Inadequate study** of carcinogenic activity describes studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

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

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

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the NTP draft Technical Report on C.I. Acid Red 114 on March 11, 1991 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 in reviewing NTP studies:

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

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SUMMARY OF PEER REVIEW COMMENTS

On March 11, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of C.I. Acid Red 114 received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Committee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J. K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of C.I. Acid Red 114 by noting this was one of five chemicals being evaluated as part of the NTP Benzidine Dye Initiative, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplasms and nonneoplastic lesions in male and female rats. The proposed conclusions were *clear evidence of carcinogenic activity* for male and female F344/N rats.

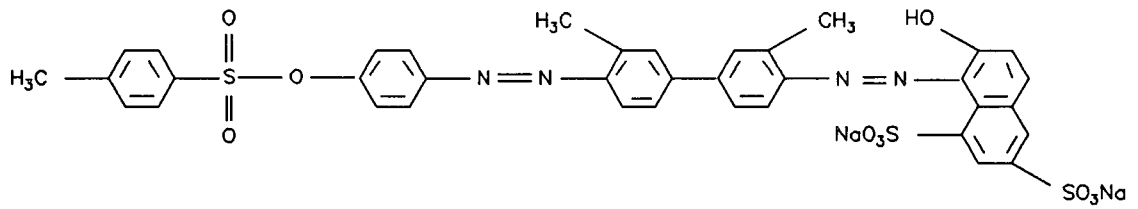
Dr. Zeise, a principal reviewer, agreed in principle with the conclusions. However, she proposed that increased incidences of mononuclear cell leukemia and thyroid follicular cell neoplasms in female rats may have been related to chemical administration and should be cited as such in the conclusions.

Dr. McKnight, the second principal reviewer, agreed with the conclusions. She also thought that the increased incidences of mononuclear cell leukemia and thyroid follicular cell neoplasms in female rats may have been related to chemical administration. In response to Drs. Zeise and McKnight, Dr. Dunnick stated that there was not enough evidence to support even a marginal finding for thyroid tumors in that there was an increase only in the low-dose group, no increase by the trend test, and no increase in precursor hyperplastic lesions in dosed groups. With regard to mononuclear cell leukemia in female rats, Dr. Dunnick commented that incidences in dosed groups were within the historical control range and that high and early mortality in the high-dose group was felt to be due to toxicity of the chemical and not mononuclear cell leukemia. Dr. McKnight pointed out that by the life table test, the test normally used for mononuclear cell leukemia, the pairwise comparison of each of the dose groups with the control group is statistically significant, and the trend test is highly significant.

Dr. Davis, the third principal reviewer, agreed with the conclusions. He asked why the doses in female rats were double those in males since hematologic data and data on kidney degeneration from the 13-week studies suggested females were more sensitive to toxic effects. Dr. Dunnick said apparent liver toxicity in males in the 13-week studies was the primary reason for the different dose levels used in 2-year studies.

Dr. Zeise moved that the Technical Report on C.I. Acid Red 114 be accepted with the revisions discussed and the conclusions as written for male and female rats, *clear evidence of carcinogenic activity*, with the addition of mononuclear cell leukemia to the conclusion for female rats as "may have been related to chemical administration." Dr. McKnight seconded the motion, which was accepted unanimously with ten votes.

INTRODUCTION



C.I. ACID RED 114

CAS No. 6459-94-5

Chemical Formula: $C_{37}H_{28}N_4O_{10}S_3Na_2$ Molecular Weight: 830.8

Synonyms: 1,3-Naphthalenedisulfonic acid, 8-((3,3′-dimethyl-4′-((4-(((4-methylphenyl)sulfonyl)oxy)phenyl)azo)(1,1′-biphenyl)-4-yl)azo)-7-hydroxy, disodium salt, Acid Leather Red BG, Acid Red 114, Amacid Milling Red PRS, Benzyl Fast Red BG, Benzyl Red BR, Cerven Kysela, C.I. 23635, Erionyl Red RS, Folan Red B, Kayanol Milling Red RS, Leather Fast Red B, Levanol Red GG, Midlon Red PRS, Milling Red B, Milling Red BB, Milling Red SWB, NCI C61096, Polar Red RS, Sandolan Red N-RS, Sella Fast Red RS, Sulphonol Fast Red R, Supranol Fast Red GG, Supranol Red PBX-CF, Supranol Red R, Telon Fast Red GG, Tertracid Milling Red B, Vondamol Fast Red RS

PRODUCTION, USE, AND EXPOSURE

C.I. Acid Red 114, an azo dye, is a red powder that decomposes between 250° C and 300° C. It is produced by coupling 2 moles of phenol to *o*-toluidine (3,3′-dimethylbenzidine) followed by coupling this precursor to G acid (2-naphthol-6,8-disulfonic acid) (*Kirk-Othmer*, 1978).

Azo dyes based on benzidine and benzidine congeners (3,3′-dimethylbenzidine and 3,3′-dimethoxybenzidine) constitute a group of over 90 dyes, all widely used in the United States. The United States Environmental Protection Agency (USEPA) reports there are six manufacturers and two importers of C.I. Acid Red 114 (USEPA, 1988). Although annual production volumes are listed as confidential for one of the manufacturers and for both importers, the remaining manufacturers reported collectively that production volumes ranged from 20,000 to 200,000 pounds. The most recent production volume data from the United States International Trade Commission (USITC) show that 380,000 pounds of C.I. Acid Red 114 were produced in 1979 (USITC, 1980). In 1980, 21,497 pounds of the dye were imported (USITC, 1981); the USITC did not report domestic production volumes of C.I. Acid Red 114 for 1985 or 1986 (USITC, 1986, 1987).

From a survey conducted from 1981-1983, the National Institute for Occupational Safety and Health (NIOSH) has estimated that a total of 18,510 workers may be exposed to C.I. Acid Red 114 (NIOSH, 1991). Industrial exposure to these dyes may occur through inhalation of dust or mist, through accidental ingestion, or from direct contact to the skin. The general public may be exposed to C.I. Acid Red 114 from clothes or other products containing the dye or from contaminated water supplies (USEPA, 1980; Fishbein, 1981; NIOSH, 1983).

METABOLISM

Reductive metabolism of 3,3′-dimethylbenzidine-based dyes produces 3,3′-dimethylbenzidine. Azo reduction can occur either in the liver via hepatic enzymes or in the gut by action of azo reductase associated with intestinal bacterial flora. Because highly polar compounds are absorbed from the gut with difficulty, mammals are not expected to absorb the water-soluble sulfonated dyes (Walker, 1970). Thus, reductive cleavage of benzidine-congener azo dyes is thought to occur primarily by bacterial action in the intestinal tract (Martin and Kennelly, 1981; Cerniglia *et al.*, 1982; Brown and Dietrich, 1983; Bos *et al.*, 1984, 1986). Following reductive cleavage, the less polar metabolites are subject to intestinal absorption and further metabolism by the liver.

Metabolism of 3,3′-dimethylbenzidine-based dyes to 3,3′-dimethylbenzidine occurs in dogs and rats (Lynn *et al.*, 1980) and also in humans (NIOSH, 1981). Following exposure to C.I. Acid Red 114, 3,3′-dimethylbenzidine was detected in the urine of dogs and rats (Lynn *et al.*, 1980). Dogs metabolized the dyes Direct Blue 25 and Acid Red 114 to 3,3′-dimethylbenzidine and excreted it in urine. Rats metabolized Direct Blue 25 to 3,3′-dimethylbenzidine and *N*-acetyl-3,3′-dimethylbenzidine, with urine concentrations of 3,3′-dimethylbenzidine comparable to those observed for dogs. However, rats given Acid Red 114 excreted only trace amounts of 3,3′-dimethylbenzidine in urine.

NIOSH (1980) reported the presence of 3,3′-dimethylethylbenzidine in the urine of two employees working in a dye manufacturing plant. The workers were in contact with 3,3′-dimethylbenzidine-based dyes, but not with 3,3′-dimethylbenzidine itself. The presence of 3,3′-dimethylbenzidine in the urine may have resulted from metabolism of the dyes or from exposure to dyes contaminated with 3,3′-dimethylbenzidine. Hartman *et al.* (1978) found that a cell-free extract of *Fusobacterium*, a human intestinal anaerobe, reduced Trypan Blue (C.I. Direct Blue 14), a 3,3′-dimethylbenzidine-derived dye, to 3,3′-dimethylbenzidine.

Tanaka *et al.* (1982) reported that urine extracts from rats treated with 3,3′-dimethylbenzidine or Evans Blue, a 3,3′-dimethylbenzidine-derived dye, contained *N*-acetyl-3,3′-dimethylbenzidine and *N,N*′-diacetyl-dimethylbenzidine, as well as 3,3′-dimethylbenzidine. Urine extracts containing these metabolites were more mutagenic than those containing only 3,3′-dimethylbenzidine. Although Evans Blue was not mutagenic, urine extracts from rats exposed to Evans Blue were mutagenic.

REPRODUCTIVE TOXICOLOGY

Wilson (1955) studied the teratogenic potential of several benzidine-based dyes in albino rats by injecting pregnant females with a 1% aqueous solution of each dye on days 7, 8, and 9 of pregnancy. Trypan Blue was the most potent teratogen, causing malformations in 49% of living offspring compared to 0% in untreated albino rats, followed by Evans Blue, which caused 14% abnormalities, Niagara Blue 4B (C.I. Direct Blue 15), which caused 4% abnormalities, and Niagara Sky Blue 6B, which caused 3% abnormalities. The teratogenic effects of the azo dyes were confirmed in a series of studies by Beaudoin and Pickering (1960), Lloyd *et al.* (1965), Beck and Lloyd (1966), Lloyd and Beck (1966), and Beaudoin (1968). Although the purity and chemical characterization of the dyes used were not reported, the abnormalities were generally similar to common spontaneous malformations such as anencephaly, hydrocephaly, and spina bifida.

TOXICITY AND CARCINOGENICITY STUDIES OF RELATED COMPOUNDS

In 1980, NIOSH and the OSHA issued a health hazard alert stating that persons working with 3,3′-dimethoxybenzidine-, benzidine-, or 3,3′-dimethylbenzidine-based dyes should be aware of the potential health hazards associated with excess exposure (NIOSH, 1981). In a later report issued to alert workers to the hazards of benzidine congener dyes, NIOSH stated that workplace exposure to dyes based on 3,3′-dimethoxybenzidine may pose a carcinogenic risk to workers (NIOSH, 1983). These conclusions were based on evidence from animal studies indicating that 3,3′-dimethoxybenzidine is carcinogenic and on evidence that dyes based on 3,3′-dimethoxybenzidine may be metabolized to the parent compound.

No epidemiologic data on the occurrence of cancer in workers exposed to either C.I. Acid Red 114 or 3,3′-dimethylbenzidine in the absence of other suspected carcinogens were found in the literature.

Benzidine

C.I. Acid Red 114 is a benzidine congener-based dye. Benzidine is a known carcinogen for humans (Scott, 1952; Case *et al.*, 1954; IARC, 1972a,b; Zavon *et al.*, 1973), rats (Spitz *et al.*, 1950; Griswold *et al.*, 1968), hamsters (Saffiotti *et al.*, 1966), and mice (Bonser *et al.*, 1956; Prokofjeva, 1971; IARC, 1972a,b; Frith and Dooley, 1976). Occupational exposure to benzidine for up to 30 years resulted in urinary bladder tumors in as many as 90% of the workers studied (Scott, 1952). Exposure to benzidine may occur directly or by reductive metabolism of benzidine-based dyes. Several reviews address the carcinogenicity of benzidine extensively (IARC, 1972a,b; Haley, 1975; USEPA, 1980; IARC, 1982).

Benzidine exposure caused urinary bladder tumors in dogs (Spitz *et al.*, 1950); hepatocellular, harderian gland, and lymphoreticular tumors in mice (Bonser *et al.*, 1956; Vesselinovitch *et al.*, 1975; Frith and Dooley, 1976); Zymbal's gland, hepatocellular, and mammary gland carcinomas in rats (Spitz *et al.*, 1950; Griswold *et al.*, 1968); and hepatocellular carcinomas, adenomas, and cholangiomas in hamsters (Saffiotti *et al.*, 1967). Animal survival was poor in many of the carcinogenicity studies of benzidine. Although in most cases this was due to the administration of toxic doses, these studies demonstrate that benzidine is carcinogenic in laboratory animals.

3,3′-Dimethylbenzidine

3,3′-Dimethylbenzidine, a methylated congener of benzidine, has been shown to be carcinogenic in rats. In early studies, Spitz *et al.* (1950) demonstrated the ability of the compound to induce Zymbal's gland neoplasms in rats. In a series of experiments, 3,3′-dimethylbenzidine dihydrochloride administered subcutaneously to rats was shown to cause neoplasms of the Zymbal's gland, small intestine, and mammary gland (Pliss, 1963, 1965; Pliss and Zabezhinsky, 1970). From a review of the literature, IARC (1972b) concluded that 3,3′-dimethylbenzidine was a systemic carcinogen for rats when given subcutaneously. In dosed water studies with rats, 3,3′-dimethylbenzidine dihydrochloride caused neoplasms of the skin, Zymbal's gland, preputial and clitoral glands, oral cavity, intestine, liver, brain, and lung in male and female rats, as well as in the mammary gland and hematopoietic system in female rats (NTP, 1991)(Table 1).

BALB/c mice given 3,3′-dimethylbenzidine dihydrochloride in the drinking water at doses up to 140 ppm for 116 weeks showed no evidence of dose-related neoplasms in female mice, but dose-related lung neoplasms were found in male mice (Schieferstein *et al.*, 1989).

3,3′-Dimethoxybenzidine

Repeated exposure to 3,3′-dimethoxybenzidine, the metabolite of C.I. Direct Blue 15, was shown to cause neoplasms of the gastrointestinal tract, Zymbal's gland, skin, and mammary gland of rats and hamsters (Pliss, 1963, 1965; Saffiotti *et al.*, 1967; Hadidian *et al.*, 1968). Although these early studies provided evidence that 3,3′-dimethoxybenzidine is carcinogenic, the use of small numbers of animals, high toxic doses, and poor animal survival weakened the strength of this evidence.

Pliss (1963, 1965) administered 30 mg 3,3′-dimethoxybenzidine in sunflower oil by gavage to rats three times per week. Because of poor survival, this dose was reduced to 15 mg after 3 weeks; administration of this lower dose was continued for 13 months. Of the 42 rats that began the study, 18 survived through month 14. Two of the 18 survivors had neoplasms of the Zymbal's gland; none of the 50 control rats developed neoplasms at this site.

In a life span study, Saffiotti *et al.* (1967) fed diets containing 1,000 ppm 3,3′-dimethoxybenzidine to 30 male and 30 female Syrian golden hamsters. After 144 weeks of exposure, the only neoplastic finding was a transitional cell carcinoma of the urinary bladder in one animal. Sellakumar *et al.* (1969) conducted a similar study in which a higher dietary concentration of 3,3′-dimethoxybenzidine (10,000 ppm) was administered to hamsters. Forestomach papillomas were detected in 37% of the exposed animals and in 2% of the controls, but no urinary bladder lesions were detected. The latter publication is an abstract and does not detail the experimental design or survival data. Hadidian *et al.* (1968) administered 0.1, 0.3, 1.0, 3.0, 10, or 30 mg 3,3′-dimethoxybenzidine per animal per day, 5 days per week, by gavage to groups of three male and

three female F344/N rats (14 males and 15 females in the 10 mg group). The vehicle was a proprietary mixture composed of sodium chloride, sodium carboxymethylcellulose, polysorbate 80, and benzyl alcohol in water. The animals were exposed for 52 weeks, observed for an additional 6 months, and then necropsied. Although neoplasms occurred as early as day 293, most were detected at terminal necropsy. A variety of neoplasms was reported, and pooled results for all dosed male and female groups included neoplastic lesions of the urinary bladder (two papillomas), mammary gland (three carcinomas, two fibroadenomas), skin (five carcinomas), intestinal tract (three carcinomas), and Zymbal's gland (eight carcinomas). The incidence of neoplasms was significantly increased over that of the 360 pooled vehicle and untreated control rats.

The NTP dosed water studies of 3,3′-dimethoxybenzidine dihydrochloride in rats have been reported (NTP, 1990). This chemical caused neoplasms of the skin, Zymbal's gland, preputial and clitoral glands, oral cavity, intestine, and liver, as well as mesotheliomas and neoplasms in the brain of males and neoplasms in the mammary gland and uterus of females (Table 1).

BALB/c mice were given 3,3′-dimethoxybenzidine in drinking water at doses up to 630 ppm. There was a decrease in body weight gain at the 630 ppm dose level relative to the controls, but there was no evidence of treatment-related neoplasms in either sex (Schieferstein *et al.*, 1990).

C.I. Direct Blue 15

The NTP rat dosed-water studies of C.I. Direct Blue 15, a benzidine dye derived from 3,3′-dimethoxybenzidine, have been reported (NTP, in press). This chemical caused neoplasms of the skin, Zymbal's gland, preputial and clitoral gland, oral cavity, large intestine, liver, brain, and uterus as well as mononuclear cell leukemia (Table 1).

o-Anisidine

o-Anisidine (2-methoxyaniline) is structurally analogous to one half of the 3,3′-dimethoxybenzidine molecule and is used to manufacture monoazo dyes by diazotization and coupling with other aromatic amines (Noller, 1965). The National Cancer Institute (NCI) found in bioassays that *o*-anisidine was carcinogenic to F344/N rats and B6C3F$_1$ mice (NCI, 1978). Groups of 55 animals of each species and sex received *o*-anisidine in feed at 5,000 or 10,000 ppm for rats and 2,500 or 5,000 ppm for mice for 103 weeks. Controls consisted of 55 untreated animals of each sex and species. Treatment with *o*-anisidine resulted in transitional cell carcinomas or papillomas of the urinary bladder in both sexes of each species. Male rats also exhibited transitional cell carcinomas of the renal pelvis and follicular cell tumors of the thyroid gland. Only one animal in any of the control groups had a urinary system tumor, which was a transitional cell papilloma of the renal pelvis in a male mouse.

o-Toluidine

o-Toluidine hydrochloride (2-aminotoluene) is structurally analogous to one half of the 3,3′-dimethylbenzidine molecule. In studies performed by NCI (1979), *o*-toluidine hydrochloride was fed to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex for 101 to 104 weeks. The feed contained concentrations of 3,000 or 6,000 ppm for rats and 1,000 or 3,000 ppm for mice. Controls consisted of 20 untreated animals of each sex and species. Exposure of rats to *o*-toluidine hydrochloride resulted in sarcomas of the spleen and other organs in both males and females, mesotheliomas of the abdominal cavity or scrotum in males, and transitional cell carcinomas of the urinary bladder in females. Administration of *o*-toluidine also resulted in increased incidences of fibromas of the subcutaneous tissue in males and fibroadenomas or adenomas of the mammary gland in females. In mice, hemangiosarcomas occurred at various sites in males, and hepatocellular carcinomas or adenomas of the mammary gland occurred in females.

GENETIC TOXICOLOGY

Information regarding the genotoxicity of C.I. Acid Red 114 is limited. The available data from the testing of metabolites of C.I. Acid Red 114 and structurally related dyes corroborate the mutagenicity of C.I. Acid Red 114 by azoreduction and release of active metabolites. As with most benzidine congener dyes, a clearly positive response in the *Salmonella typhimurium* gene mutation assay is dependent on conditions which allow metabolism of the azo bonds to release the parent amine. In

standard *S. typhimurium* assays, only weak mutagenic activity is detected with S9, primarily in frameshift strain TA98, and no activity is apparent in the absence of S9 (Venturini and Tamaro, 1979; Mortelmans *et al.*, 1986). However, when reductive metabolism precedes incubation with the *S. typhimurium* frameshift strains (TA98, TA1538) in the presence of S9, a strong mutagenic response is obtained (Elliot and Gregory, 1980; Prival *et al.*, 1984; Reid *et al.*, 1984a,b).

In mammalian cell systems, C.I. Acid Red 114 has not been tested in protocols allowing reductive metabolism. There was no induction of gene mutations in mouse L5178Y lymphoma cells with and without rat liver S9 (Rudd *et al.*, 1983), or unscheduled DNA synthesis in F344/N rats hepatocytes *in vitro* or *in vivo* (Mirsalis *et al.*, 1983). A key metabolite of C.I. Acid Red 114, 3,3′-dimethylbenzidine, was positive in a variety of *in vivo* genotoxicity assays (NTP, 1991). In the presence of S9 metabolic activation, 3,3′-dimethylbenzidine induced gene mutations in frameshift-sensitive *S. typhimurium* strains TA98 and TA1538 (Shimizu and Takemura, 1976; Hartman *et al.*, 1978; Martin and Kennelly, 1981; Waalkens *et al.*, 1981; Haworth *et al.*, 1983; Reid *et al.*, 1984a,b). Positive results with and without S9 were also obtained for induction of triflurothymidine resistance in mouse L5178Y lymphoma cells (Mitchell *et al.*, 1988; Myhr and Caspary, 1988) as well as sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells (Galloway *et al.*, 1987). 3,3′-Dimethylbenzidine was also positive for induction of unscheduled DNA synthesis (Martin *et al.*, 1978) and DNA repair (Kornbrust and Barfknecht, 1984) in mammalian cells *in vitro* with S9. Two metabolites of 3,3′dimethylbenzidine, *N*-acetyl-3,3′-dimethylbenzidine and *N,N′*-diacetyl-3,3′-dimethylbenzidine, were both positive in *S. typhimurium* strains TA98, TA100, and TA1538 in the presence of S9 (Tanaka *et al.*, 1982; Kennelly *et al.*, 1984; Reid *et al.*, 1984a,b). Benzidine, the parent compound in this series of substituted biphenyls, is positive for induction of gene mutations in *S. typhimurium* with S9 (Haworth *et al.*, 1983; Reid *et al.*, 1984b). Positive induction of micronuclei, sister chromatid exchanges, and chromosomal aberrations was also obtained in bone marrow cells of mice exposed by intraperitoneal injection (NTP, unpublished data).

STUDY RATIONALE

Benzidine is a known human carcinogen (IARC, 1972a,b; 1987a,b), and the benzidine congeners, 3,3′-dimethylbenzidine and 3,3′-dimethoxybenzidine, are known animal carcinogens (IARC, 1972b, 1974). Benzidine and benzidine congener-based dyes have been shown to be metabolized to these parent amines (Rinde and Troll, 1975; NCI, 1978; Lynn *et al.*, 1980; Nony *et al.*, 1980; Bowman *et al.*, 1982).

The National Toxicology Program's (NTP) Benzidine Dye Initiative is a collaborative effort of NIEHS, NCTR, NIOSH, USEPA, OSHA, and CPSC under the aegis of the NTP. The objective of the Initiative was to develop an integrated body of scientific data concerning the metabolism and pharmacokinetics, genetic toxicology, and *in vivo* carcinogenicity of dyes derived from benzidine, 3,3′-dimethylbenzidine, and 3,3′-dimethoxybenzidine. Because studying each of the hundreds of benzidine-based dyes was considered impractical, the research program was designed to evaluate representative benzidine congeners, benzidine congener-derived dyes, and benzidine-derived dyes.

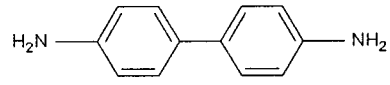
The five benzidine dyes selected for toxicity and carcinogenicity studies were: 3,3′-dimethylbenzidine and 3,3′-dimethoxybenzidine which are benzidine congeners; C.I. Acid Red 114 which is a 3,3′-dimethylbenzidine-based dye; and C.I. Direct Blue 15 and C.I. Direct Blue 218 which are 3,3′-dimethoxybenzidine-based dyes (Figure 1).

The oral route of administration was selected for these studies to mimic potential exposure in the workplace and in the home. Because long-term studies of 3,3′-dimethylbenzidine dihydrochloride and 3,3′-dimethoxybenzidine dihydrochloride were being conducted on mice at NCTR, the NTP 2-year studies of these chemicals used only rats. Results from the studies with 3,3′-dimethoxybenzidine dihydrochloride, 3,3′-dimethylbenzidine dihydrochloride, and C.I. Direct Blue 15 have been reported (NTP, 1990, 1991, in press). Auxiliary studies by Maronpot *et al.* (1988) and Ulland *et al.* (1989) report on transplantation studies of tumors and oncogene activation (Reynolds *et al.*, 1990).

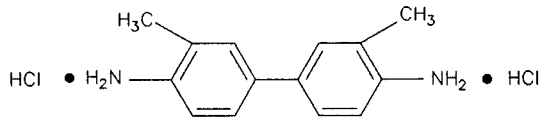
TABLE 1
Incidences of Neoplasms in National Toxicology Program Benzidine Dye Studies

Male F344/N Rats	Female F344/N Rats
Neoplasms in the 21-Month Drinking Water Studies of 3,3′-Dimethoxybenzidine Dihydrochloride[a]	
Skin basal cell or sebaceous gland neoplasms: 2/60, 33/45, 56/75, 41/60	Skin basal cell neoplasms: 0/60, 4/45, 3/75, 2/60
Skin squamous cell neoplasms: 0/60, 13/45, 28/75, 22/60	
Zymbal's gland neoplasms: 0/59, 10/45, 25/75, 30/60	Zymbal's gland neoplasms: 1/60, 12/45, 21/75, 16/60
Preputial gland neoplasms: 16/60, 12/43, 33/73, 29/59	Clitoral gland neoplasms: 7/58, 27/44, 48/74, 41/55
Palate or tongue neoplasms: 1/60, 8/45, 10/75, 11/60	Palate or tongue neoplasms: 2/60, 2/45, 6/75, 5/60
Small intestine neoplasms: 0/60, 4/45, 7/75, 5/60	
Large intestine neoplasms: 0/60, 1/45, 8/75, 8/60	Large intestine neoplasms: 0/60, 1/45, 1/75, 3/60
Liver neoplasms: 1/60, 4/45, 7/74, 8/60	Liver neoplasms: 0/60, 1/44, 0/75, 3/60
Mesotheliomas: 2/60, 1/45, 7/75, 6/60	
Brain astrocytomas: 0/60, 2/44, 3/75, 1/60	
	Mammary gland adenocarcinomas: 1/60, 2/45, 14/75, 20/60
	Uterus or cervix neoplasms: 0/60, 4/45, 2/75, 2/60
Neoplasms in the 15-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride[b]	
Skin basal cell neoplasms: 0/60, 11/45, 54/75, 30/60	Skin basal cell neoplasms: 0/60, 3/45, 10/75, 9/60
Skin sebaceous cell adenoma: 0/60, 0/45, 7/75, 5/60	
Skin keratoacanthomas: 1/60, 1/45, 8/75, 5/60	
Skin squamous cell neoplasms: 0/60, 2/45, 17/75, 27/60	Skin squamous cell neoplasms: 0/60, 3/45, 9/75, 12/60
Zymbal's gland neoplasms: 1/59, 3/45, 32/75, 36/59	Zymbal's gland neoplasms: 0/57, 6/44, 32/73, 42/60
Preputial gland neoplasms: 2/60, 4/45, 6/75, 9/60	Clitoral gland neoplasms: 0/60, 14/45, 42/75, 32/59
Liver neoplasms: 0/60, 0/45, 35/75, 33/60	Liver neoplasms: 0/60, 0/45, 7/74, 4/60
Oral cavity neoplasms: 0/60, 0/45, 4/75, 5/60	Oral cavity neoplasms: 0/60, 3/45, 9/75, 13/60
Small intestine neoplasms: 0/60, 0/45, 4/75, 8/60	Small intestine neoplasms: 0/60, 1/45, 3/75, 5/60
Large intestine neoplasms: 0/60, 0/45, 6/75, 15/60	Large intestine neoplasms: 0/60, 1/45, 7/75, 4/60
	Mammary gland adenocarcinoma: 0/60, 1/45, 3/75, 6/60
Lung neoplasms: 1/60, 0/45, 8/75, 6/60	Lung neoplasms: 1/60, 1/45, 3/74, 4/60
Mesothelioma: 0/60, 0/45, 3/75, 4/60	
Brain neoplasms: 0/60, 0/45, 1/75, 2/60	Brain neoplasms: 0/60, 2/45, 2/75, 1/60
	Mononuclear cell leukemia: 1/60, 3/45, 6/75, 4/60
Neoplasms in the 22-Month Drinking Water Studies of C.I. Direct Blue 15[c]	
Skin basal cell neoplasms: 2/50, 9/35, 27/65, 28/50	
Skin sebaceous cell adenoma: 0/50, 1/35, 7/65, 3/50	
Skin keratoacanthomas: 2/50, 1/35, 7/65, 2/50	
Skin squamous cell neoplasms: 2/50, 4/35, 11/65, 19/50	Skin squamous cell neoplasms: 0/50, 2/35, 6/65, 5/50
Zymbal's gland neoplasms: 1/50, 5/35, 10/65, 20/50	Zymbal's gland neoplasms: 0/50, 4/35, 11/65, 17/50
Preputial gland neoplasms: 8/49, 5/35, 23/64, 9/48	Clitoral gland neoplasms: 7/50, 11/31, 24/64, 27/50
Liver neoplasms: 0/50, 6/35, 9/65, 11/50	Liver neoplasms: 0/50, 0/35, 2/65, 5/50
Oral cavity neoplasms: 1/50, 10/35, 24/65, 17/50	Oral cavity neoplasms: 2/50, 4/35, 19/65, 15/50
Small intestine neoplasms: 0/50, 1/35, 0/65, 2/50	Small intestine adenocarcinoma: 0/50, 0/35, 1/65, 3/50
Large intestine neoplasms: 0/50, 1/35, 6/65, 8/50	Large intestine adenomatous polyp: 0/50, 0/35, 3/65, 1/50
	Uterine neoplasms: 1/50, 0/35, 1/65, 4/50
Mononuclear cell leukemia: 17/50, 19/35, 28/65, 20/50	Mononuclear cell leukemia: 7/50, 13/35, 27/65, 15/50
Brain neoplasms: 0/50, 1/35, 1/65, 2/50	

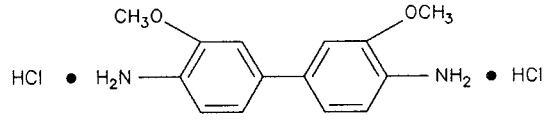
[a] Dose groups: 0, 80, 170, 330 ppm
[b] Dose groups: 0, 30, 70, 150 ppm
[c] Dose groups: 0, 630, 1,250, 2,500 ppm



Benzidine
CAS No. 92-87-5



3,3'-Dimethylbenzidine Dihydrochloride
CAS No. 612-82-8



3,3'-Dimethoxybenzidine Dihydrochloride
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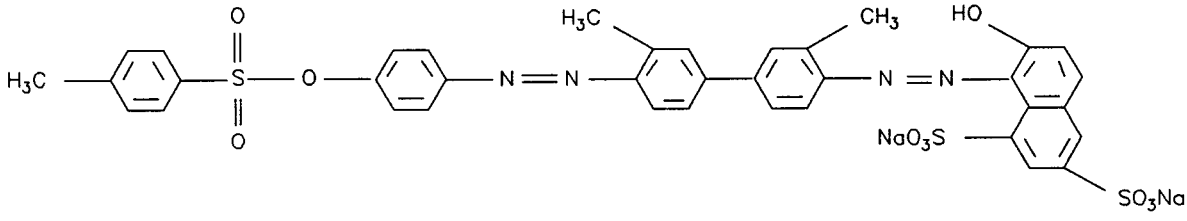
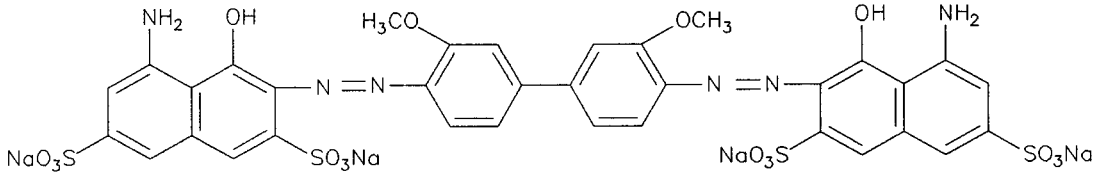
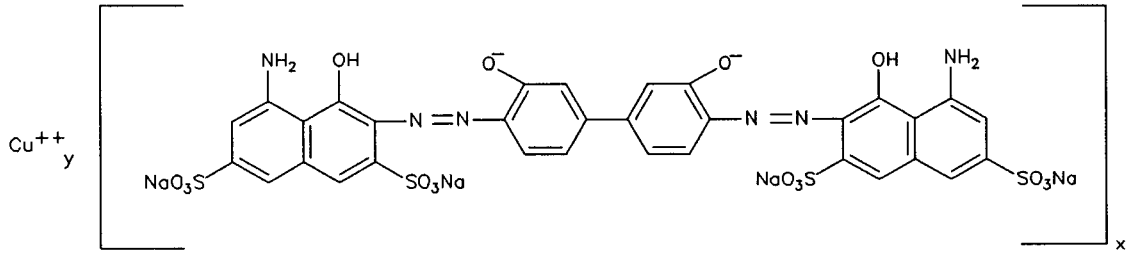


C.I. Acid Red 114
CAS No. 6459-94-5



C.I. Direct Blue 15
CAS No. 2429-74-5



C.I. Direct Blue 218
CAS No. 28407-37-6

FIGURE 1
Chemical Structure of Benzidine and Selected Benzidine Congeners and Dyes

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF C.I. ACID RED 114

C.I. Acid Red 114 was obtained in one lot (A101681) from the Atlantic Chemical Company, Nutley, NJ. The dye was desalted in two batches designated as Lots M113081 and M032582. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (MRI), Kansas City, MO (Appendix F). Lot M113081 was used in the 14-day drinking water studies, and Lot M032582 was used in the 13-week and 2-year drinking water studies.

The study dye, a red powder, was identified as C.I. Acid Red 114 by infrared and nuclear magnetic resonance spectroscopy (Appendix F). Purity was evaluated by elemental analysis, water analysis, titration of azo groups, spark source mass spectroscopy, thin layer chromatography (TLC), and high performance liquid chromatography (HPLC). Percent purity was estimated as 82% for Lot M113081 and 85% for Lot M032582.

There were approximately 15 organic impurities observed by HPLC analysis; these impurities were similar in structure to the major component, with the two largest components estimated at 3%. In addition, there was approximately 1% to 4% water and 1% sodium chloride present in the dye. The level of benzidine detected by HPLC did not exceed 1 ppm in either lot; whereas 3,3'-dimethylbenzidine was detected in both lots at a concentration of approximately 5 ppm.

The dye was found to be stable in bulk form when stored protected from light for at least 2 weeks at temperatures up to 60° C. Based on the stability study results, the bulk chemical was stored at room temperature in the dark at the study laboratory. The stability of the bulk chemical was monitored by the study laboratory by HPLC and ultraviolet/visible spectroscopy. No degradation of the study material was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing the appropriate amount of C.I. Acid Red 114 with tap water (Fairfax Co., VA) for the 13-day studies, or with distilled water (Polar Water Co., Beltsville, MD) for the 13-week and 2-year studies. Stability studies conducted by the analytical chemistry laboratory showed that solutions of 375 ppm C.I. Acid Red 114 in water were stable for at least 3 weeks in the dark at room temperature and for at least 3 days under simulated animal room conditions. Dose formulations were prepared twice weekly for the 14-day and 13-week studies. Dose formulations were prepared in a similar manner for the 2-year studies but were stored 1 week at room temperature prior to use. Preparation and storage procedures for dosed drinking water in the studies of C.I. Acid Red 114 are presented in Appendix F.

Dose formulations were routinely sampled to determine concentration throughout all three studies. In the 13-day studies, dose formulations from the animal room were analyzed prior to test initiation and at the end of the studies (Table F2). In the 13-week studies, dose formulations were analyzed prior to initiation of the studies, at mid-point, and at study end (Table F3). In the 2-year studies, dose formulations were analyzed prior to initiation and at least once every four weeks for the duration of the studies (Table F4). Samples were taken for analysis from the animal room throughout the 13-week and 2-year studies. While the concentrations of the high-dose formulations used in the 13-day studies were outside the ± 10% specifications, all formulations for the 13-week and 2-year studies were within 10% of the target concentrations. Dose formulations were sampled for periodic referee analyses by the analytical laboratory, and results from both laboratories were in agreement (Table F5).

13-DAY STUDIES

Male and female F344/N rats were obtained from Frederick Cancer Research Center (Frederick, MD)

and observed for 14 days prior to the studies. The rats were 7 weeks old when treatment was initiated.

Animals of each sex were weighed and assigned to a weight class; then five animals were randomly placed into each of the dose groups. Groups of five rats of each sex received either 0, 10,000, 20,000, or 30,000 ppm C.I. Acid Red 114 in drinking water for 13 consecutive days. Feed and water were supplied *ad libitum*. Animals were observed twice daily for mortality and once daily for clinical signs. Water consumption by cage was measured twice weekly but recorded as a weekly total. Body weights were measured three times: at the initiation of treatment, at day 7, and at day 13. Complete necropsies were performed on all animals at the end of the studies. The following organs were removed and weighed: brain, heart, liver, lung, right kidney, right testis (males), and thymus. Ratios of organ weight to body weight were determined. Complete histopathologic examinations were performed on all control and the 30,000 ppm dose group animals. In addition, the sternebrae, marrow, and thymus were examined from males and females in 10,000 and 20,000 ppm dose groups. Further experimental details are presented in Table 2.

13-Week Studies

The 13-week studies were conducted to evaluate cumulative toxic effects of repeated exposure to C.I. Acid Red 114 and to determine dose levels for the 2-year studies. The strain and source of rats were the same as the 13-day studies. Rats were randomly assigned by weight class to dose groups and were caged as described for the 13-day studies (Table 2). Rats were observed for 15 days prior to initiation of the studies and were 7 to 8 weeks old when the studies began.

Groups of 10 F344/N rats of each sex received 0, 600, 1,200, 2,500, 5,000, or 10,000 ppm C.I. Acid Red 114 in drinking water. The dosing period was 94 days for males and 95 days for females. Feed and water were supplied *ad libitum*. Animals were observed twice daily for mortality and once daily for clinical signs. Feed and water consumption were measured as in the 13-day studies. Body weights were measured at study initiation and then weekly for the duration of the studies. Complete necropsies were performed on all animals. The age at necropsy was 20 to 21 weeks. Final body weights, selected organ weights, and organ-weight-to-body-weight ratios were measured as in the 13-day studies (Table 2). Complete histopathologic examinations were performed on all control animals and the 10,000 ppm dose group. Organs examined in the lower dose groups were: liver, spleen, mesenteric lymph node, pancreas, and right kidney (females only).

Hematology and clinical chemistry evaluations were performed on blood samples drawn from the abdominal aorta of all animals surviving to the end of the studies. Hematology and clinical chemistry analyses are listed in Table 2.

2-Year Studies

Study Design

C.I. Acid Red 114 was administered in distilled drinking water for 104 weeks. Rats were separated by sex, weighed and grouped by weight class and assigned allocation according to the recommendations of Portier and Hoel (1984). The numbers of animals placed on study for each dose group were 70 controls, 45 low-dose, 75 mid-dose, and 70 high-dose. Male rats received 0, 70, 150, or 300 ppm C.I. Acid Red 114; female rats received 0, 150, 300, or 600 ppm.

Source and Specification of Animals

Strain and species of rats were obtained from the same source as for the 13-day and 13-week studies. The animals were 4 weeks old when received. They were observed for 9 days prior to treatment and were 5 weeks old at the initiation of these studies. Ten animals, five of each sex, were randomly selected and sacrificed prior to the studies and examined for parasites or signs of disease. Serum samples were collected for viral screens. Animal health was monitored throughout the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

Animal Maintenance

Animals were housed five per cage as in the other studies. Cages were rotated on the racks from top to bottom and racks of cages within the animal room were rotated every two weeks. Feed and water were supplied *ad libitum*. Distilled water (Polar Water Co., Beltsville, MD) was the vehicle used in administering the chemical to the dosed

animals or the controls. Feed composition is presented in Appendix H. Additional details on animal maintenance are given in Table 2.

Clinical Observations and Pathology

All animals were observed twice daily. Clinical findings were recorded during body weight measurements. Body weights were recorded weekly for the first 14 weeks, again at week 16, and then every four weeks until week 92. After week 92, weights were recorded biweekly up to week 104. Water consumption was recorded twice weekly, except for week 104, and averaged every four weeks.

Organ weights and organ-weight-to-body-weight ratios were determined for control and high-dose groups at 9 months and for all dose groups at 15 months.

Hematology parameters were measured from blood collected from the retroorbital sinus after 39 weeks of chemical exposure for the 9-month evaluation, and after 65 weeks of chemical exposure for the 15-month evaluation. Clinical chemistry analyses were performed on blood sampled from the abdominal aorta on the day of sacrifice. Animals were fasted for 16 hours and anesthetized with sodium pentobarbital just prior to blood collection. Samples for urinalysis were collected by housing ten animals in metabolism cages for 24 hours with only distilled drinking water available.

Ten animals were selected from the high-dose and control groups for the 9-month interim evaluations; an additional 10 animals were selected from each dosed and control group for the 15-month interim evaluations. Complete histologic examinations were conducted on all animals that died or were killed moribund and on all animals necropsied at the end of the studies. Also, selected tissues were evaluated from low- and mid-dose group animals from the 15-month interim evaluations (Table 2). Tissues for microscopic examination were preserved in 10% neutral buffered formalin, then embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

When the pathology evaluation was completed by the study laboratory pathologist and the pathology data entered into the Toxicology Data Management System (TDMS), the microscope slides, individual animal necropsy records, and pathology tables were forwarded to an independent pathology quality assessment laboratory. Individual animal records and pathology tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated.

A quality assessment pathologist reviewed selected tissues microscopically for accuracy and consistency of lesion diagnosis. All neoplastic and nonneoplastic lesions were reviewed in the following organs: from male rats, liver and Zymbal's gland; from female rats, liver, Zymbal's gland, lung, clitoral gland, and thyroid gland. The adrenal medulla from all males and females also was reviewed for proliferative lesions, and spleen and liver from all females were reviewed to confirm the presence of mononuclear cell leukemia. In addition, all neoplastic diagnoses in tissues other than those already mentioned were reviewed in all animals, and all diagnoses (neoplastic and nonneoplastic) were reviewed from a random 10% of the animals from each control and high-dose group.

The quality assessment report and slides were submitted to the Pathology Working Group (PWG) chair, who reviewed the slides of tissues with chemical-related effects and of any other tissues for which there was disagreement in diagnosis between the laboratory and quality assessment pathologist. Representative histopathology slides of tissues with chemical-related lesions and examples of disagreements in diagnosis between the laboratory and quality assessment pathologist were shown to the PWG. The PWG included the quality assessment pathologist and others experienced in rodent toxicologic pathology who examined the tissues without knowledge of dose group or previously rendered diagnoses. Whenever the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the diagnosis was changed to reflect the opinion of the PWG. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). The final pathology data represent a consensus of contractor pathologists and the NTP PWG. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type were separated or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead from other than natural causes. Animals dying from natural causes were not censored. Statistical analysis 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 to identify dose-related trends. All reported P values for the survival analysis are two-sided.

Calculation of Incidence

The incidence of neoplasms 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 the site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., oral cavity) prior to tissue sampling for histopathology, or when lesions (e.g., lymphomas) could have occurred at multiple sites, the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence

In the 2-year studies, the deaths of dosed rats and those killed moribund during the studies were considered due to tumors of the skin, Zymbal's gland, and clitoral gland. Consequently, for these particular lesions, primary emphasis in the analysis of tumor incidence was given to the life table test (Cox, 1972; Tarone, 1975), a survival-adjusted procedure appropriate for rapidly lethal tumors.

For incidental tumors (tumors discovered as a result of death from an unrelated cause), the primary statistical method used in this study was logistic regression, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When tumors are incidental, this comparison of the time-specific tumor prevalences also provides a comparison of the time-specific tumor incidences (McKnight and Crowley, 1984).

In some instances the reduced survival in dosed animals (due largely to the increased incidence of lethal tumors) reduced the power of logistic regression to detect carcinogenic effects. In these instances, procedures based on effective number of animals (i.e., the number of animals surviving until the appearance of the first tumor of that particular type) were given primary emphasis. These procedures include the Fisher's exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979).

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

Historical Control Data

Although the concurrent control group is the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Tumor incidences from the NTP historical control data base for 2-year studies (Haseman *et al.*, 1984, 1985) are included for tumors appearing to show compound-related effects.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the multiple comparison procedures of

Dunnett (1955) and Williams (1971, 1972). Clinical chemistry and hematology data, which have typically skewed distributions, were analyzed using the multiple comparison methods of Dunn (1964) and Shirley (1977). Jonckheere's test (Jonckheere, 1954) was used to evaluate the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response trend (Dunnett's or Dunn's test). For the 9-month studies (in which a single dose group was compared with the controls), Wilcoxon's rank sum test (Hollander and Wolfe, 1973) was used to evaluate organ weight, hematology, serum chemistry, and urinalysis data.

Quality Assurance Methods

The 13-week and 2-year studies were conducted in compliance with FDA Good Laboratory Practice Regulations (21 CFR Part 58). In addition, as study records were submitted to the NTP Archives, they were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and the preliminary draft of this NTP Technical Report were conducted. Audit procedures are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff so that all had been resolved or were otherwise addressed during the preparation of this Technical Report.

TABLE 2
Experimental Design and Materials and Methods in the Drinking Water Studies of C.I. Acid Red 114

13-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
Hazleton Laboratories America, Inc. (Vienna, VA)	Hazleton Laboratories America, Inc. (Vienna, VA)	Hazleton Laboratories America, Inc. (Vienna, VA)
Strain and Species		
F344/N rats	F344/N rats	F344/N rats
Animal Source		
Frederick Cancer Research Center (Frederick, MD)	Frederick Cancer Research Center (Frederick, MD)	Frederick Cancer Research Center (Frederick, MD)
Time Held Before Study		
14 days	15 days	9 days
Age When Placed on Study		
49 days	54 days	35 days
Date of First Dose		
30 March 1982	29 June 1982	10 June 1983
Date of Last Dose		
12 April 1982	31 October 1982 for males 1 November 1982 for females	31 May 1985
Duration of Dosing		
13 consecutive days	94 days for males 95 days for females	104 weeks (7 days/week)
Age at Necropsy		
62 days	21 weeks	109 weeks 45 weeks (9 month interim) 71 weeks (15 month interim)
Necropsy Dates		
12 April 1982	1 and 2 November 1982	10-12 June 1985
Size of Study Groups		
5 males and 5 females	10 males and 10 females	Control: 70/sex Low-dose: 45/sex Mid-dose: 75/sex High-dose: 70/sex
Method of Animal Distribution		
Animals distributed to weight classes and then randomized to test and control groups and position in racks.	Same as 13-day studies	Same as 13-day studies
Animals per Cage		
5	5	5
Method of Animal Identification		
Ear punch	Ear punch	Ear punch then ear tag

TABLE 2
Experimental Design and Materials and Methods in the Drinking Water Studies of C.I. Acid Red 114 (continued)

13-Day Studies	13-Week Studies	2-Year Studies
Diet NIH-07 Rat and Mouse Ration, meal (Zeigler Bros., Inc., Gardners, PA); available *ad libitum*	Same as 13-day studies	Same as 13-day studies
Maximum Storage Time for Feed 120 days after milling	Same as 13-day studies	Same as 13-day studies
Water Tap water (Fairfax County Water Authorities) in glass water bottles with stainless steel sippers (Hazleton Systems, Inc., Aberdeen, MD); available *ad libitum*	Distilled water (Polar Water Co., Beltsville, MD) in glass water bottles with stainless steel sippers (Hazleton Systems, Inc., Aberdeen, MD); available *ad libitum*	Same as 13-week studies
Cages Polycarbonate (Hazleton Systems, Inc., Aberdeen, MD)	Same as 13-day studies	Same as 13-day studies
Bedding Heat-treated hardwood chips (P.J. Murphy Forest Products, Mt. Jewett, PA)	Same as 13-day studies	Same as 13-day studies
Cage Filters Reemay nonwoven polyester fiber filters (DuPont Company, Applied Technologies Division, Wilmington, DE)	Same as 13-day studies	Same as 13-day studies
Animal Room Environment Temperature: 72°-78° F Relative humidity: 27%-69% Fluorescent light: 12 hours/day	Temperature: 70°-75° F Relative humidity: 25%-74% Fluorescent light: 12 hours/day Room air changes: 16/hour	Temperature: 66°-83° F Relative humidity: 25%-77% Fluorescent light: 12 hours/day Room air changes: 11.4/hour
Doses 0, 10,000, 20,000 or 30,000 ppm C.I. Acid Red 114 in drinking water	0, 600, 1,200, 2,500, 5,000, 10,000 ppm C.I. Acid Red 114 in drinking water	0, 70 (males only), 150, 300, or 600 (females only) ppm C.I. Acid Red 114 in drinking water
Type and Frequency of Observation Observed twice/day; body weight initially and once/week; water consumption twice/week; clinical observation daily	Observed twice/day; body weight initially and once/week; water consumption twice/week; clinical observation once/week	Observed twice/day; body weights initially, once/week for 14 weeks to week 16, once/month thereafter to week 92, then every 2 weeks to week 104; water consumption measured in a 3- or 4-day segment twice/week, recorded every 4 weeks; not measured at week 104; clinical observations at body weight determinations

TABLE 2
Experimental Design and Materials and Methods in the Drinking Water Studies of C.I. Acid Red 114 (continued)

13-Day Studies	13-Week Studies	2-Year Studies
Necropsy Necropsy performed on all animals. Organ weights obtained at necropsy (brain, heart, liver, lung, right kidney, right testis, and thymus). **Histopathology** Complete histopathology on male and female control and high-dose (30,000 ppm) animals, including the following tissues: adrenal gland, bone (sternebrae, including marrow), brain, clitoral gland, epididymis, esophagus, heart, kidney, large intestines (cecum, colon, rectum), liver, lymph nodes (mandibular, mesenteric), mammary gland, nasal turbinates, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, small intestines (duodenum, ileum, jejunum), spleen, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus, Zymbal's gland, and gross lesions. The following tissues were examined from 10,000 and 20,000 ppm males and females: bone (sternebrae, including marrow) and thymus. **Clinical Pathology** None required	**Necropsy** Necropsy performed on all animals. Organ weights measured were the same as in the 13-day studies. **Histopathology** Complete histopathology on male and female controls and all males and females receiving 10,000 ppm. Tissues examined were the same as in the 13-day studies complete screen. Selected tissues were examined from other dose groups as follows: 1,200, 2,500, and 5,000 ppm dose groups, liver, spleen, pancreas, mesenteric lymph node from males and females, kidney from females only; 600 ppm dose group, liver, spleen, pancreas from males and females, mesenteric lymph node from males only, kidney from females only. **Clinical Pathology** Clinical pathology studies were conducted at the end of the studies. ***Hematology:*** hematocrit, hemoglobin, erythrocytes, and leukocyte count and differential. ***Clinical chemistry:*** urea nitrogen, creatinine, alanine aminotransferase, lactate dehydrogenase, and sorbitol dehydrogenase.	**Necropsy** Necropsy performed on all animals. Organ weights measured at 9-month and 15-month interim sacrifices (brain, kidney, liver). **Histopathology** Complete histopathology on all animals from the 9-month interim evaluations, all control and high-dose animals from the 15-month interim evaluations, all animals that died or killed moribund, and all animals killed at the end of the studies. Tissues examined were the same as in the 13-day studies complete screen with the addition of seminal vesicles. In 15-month interim evaluations, tissues examined from low- and mid-dose groups were: 70 and 150 ppm males, liver, kidney, lung, preputial gland, Zymbal's gland, mesenteric lymph node; 150 and 300 ppm females, adrenal gland, liver, kidney, lung, clitoral gland, Zymbal's gland, spleen, pancreas; 300 ppm females only, mesenteric lymph node. **Clinical Pathology** Clinical pathology studies were conducted at 9 and 15 months. ***Hematology:*** hematocrit, hemoglobin, erythrocytes, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and leukocyte count and differential. ***Clinical chemistry:*** urea nitrogen, creatinine, serum glucose, alanine aminotransferase, lactate dehydrogenase, sorbitol dehydrogenase, triiodothyronine, thyroxine, thyroid-stimulating hormone, serum osmolality, and osmolality ratio. ***Urinalyses:*** urine osmolality, creatinine excretion, urine creatinine, urine volume, specific gravity, and urine pH

RESULTS

13-DAY STUDIES

All rats survived to the end of the studies except for the accidental death of one male in the 20,000 ppm dose group. Final mean body weights were significantly lower for males in the mid- and high-dose groups and for females in all dose groups (Table 3). Overall water consumption decreased with increasing dose. Significantly reduced body weights in the dosed animals made evaluation of the organ weights difficult (Table D1). Organ-weight-to-body-weight ratios were significantly increased for some organs in males and females but were considered secondary to the decrease in body weight. In males, both absolute and relative thymus weights were significantly decreased in mid- and high-dose groups. There were no notable necropsy findings, although organs and tissues were stained red in dosed animals.

Chemical-related histopathologic findings were observed in sternal bone marrow and thymus of both sexes. In males and females receiving 20,000 ppm C.I. Acid Red 114, hypocellularity of sternal bone marrow was found in three males and in all females. In these animals, the marrow was depleted of both erythroid and myeloid cells. This condition was present in only one animal of each sex in the 10,000 ppm dose group. Lymphocytic depletion of the thymus was observed in four males and one female in the 20,000 ppm dose group, but was not observed in the 10,000 ppm dose group.

TABLE 3
Survival and Mean Body Weights of Rats in the 13-Day Drinking Water Studies of C.I. Acid Red 114

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Water Consumption[c] Day 1	Day 13
Male							
0	5/5	152 ± 3	218 ± 6	66 ± 3	–	22	21
10,000	5/5	134 ± 1[d]	206 ± 2	72 ± 1	94	21	17
20,000	4/5[e]	154 ± 3	181 ± 3**	29 ± 2**	83	18	18
30,000	5/5	155 ± 3	168 ± 6**	12 ± 3**	77	15	18
Female							
0	5/5	122 ± 3	151 ± 4	29 ± 1	–	20	20
10,000	5/5	119 ± 2	139 ± 2**	20 ± 1**	92	19	13
20,000	5/5	119 ± 2	133 ± 3**	14 ± 2**	88	13	14
30,000	5/5	119 ± 1	121 ± 3**	2 ± 3**	80	12	11

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies.
[c] Grams per animal per day, based on average consumption data per group per day for days 1 and 13.
[d] A review of the data did not identify the reason for the low initial body weights for this group. At randomization, the mean body weights for all groups was 134 grams. However, 4 days later at study initiation, weights of all groups except for the 10,000 ppm group increased by about 20 grams.
[e] Accidentally killed before day 8

13-WEEK STUDIES

All rats survived to the end of the studies. Final mean body weights for all groups given 1,200 ppm and above were significantly lower than the untreated controls (Table 4). Water consumption for all dose groups was lower than controls.

Absolute and relative organ weights are presented in Table D2. Relative liver weights were significantly increased in all dosed males and females, while absolute and relative kidney weights were significantly increased in females receiving doses of 1,200 ppm and above.

Clinical findings attributed to C.I. Acid Red 114 included discolored fur, urine stains in females, and red crusts around noses in males. Red discolored fur was seen as early as week 2 in high-dose groups and was present in all dose groups by week 9. Discolored, urine-stained fur in females was observed in the two highest dose groups by week 2. Although observed in females by weeks 10 and 11, red crusts around noses were more prevalent in males beginning in week 5.

Hematology and clinical chemistry results for males and females are presented in Table E1. Hematocrit, hemoglobin, and erythrocyte counts were decreased in dosed females and the erythrocyte count was reduced at 1,500 ppm and above in males. These findings are consistent with the reduction in bone marrow cellularity observed in the 13-day studies and suggest that the chemical has a direct effect on hematopoietic cells at high-dose levels. Levels of alanine aminotransferase, lactate dehydrogenase, and sorbitol dehydrogenase were elevated, often significantly, in dosed males, while only sorbitol dehydrogenase levels were significantly elevated in dosed females. These findings are consistent with mild hepatocellular damage.

Slight chemical-related changes were seen in the liver, pancreas, and mesenteric lymph node of treated animals of both sexes and in the kidney of treated females (Table 5). All lesions were minimal to mild in severity. Liver changes in males were minimal and consisted of decreased staining intensity of the cytoplasm of centrilobular hepatocytes

TABLE 4
Survival and Mean Body Weights of Rats in the 13-Week Drinking Water Studies of C.I. Acid Red 114

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	160 ± 5	343 ± 5	183 ± 4	–	19	22
600	10/10	159 ± 3	333 ± 4	174 ± 4	97	18	19
1,200	10/10	157 ± 3	305 ± 5**	148 ± 4**	89	15	16
2,500	10/10	151 ± 4	298 ± 6**	147 ± 6**	87	14	15
5,000	10/10	159 ± 3	299 ± 5**	140 ± 5**	87	6	15
10,000	10/10	159 ± 4	291 ± 4**	132 ± 5**	85	10	14
Female							
0	10/10	115 ± 3	193 ± 4	78 ± 2	–	16	19
600	10/10	113 ± 3	187 ± 2	74 ± 3	97	19	16
1,200	10/10	110 ± 2	181 ± 2**	71 ± 2	94	11	16
2,500	10/10	116 ± 2	183 ± 3**	66 ± 3**	94	10	11
5,000	10/10	114 ± 3	179 ± 3**	65 ± 4**	92	10	12
10,000	10/10	116 ± 3	172 ± 2**	57 ± 3**	89	7	10

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Grams per animal per day, based on average consumption data per group per week for weeks 1 and 13.

TABLE 5
Incidences of Selected Treatment-Related Lesions in Rats in the 13-Week Drinking Water Studies of C.I. Acid Red 114

Dose	0 ppm	600 ppm	1,200 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	10	10	10	10	10	10
Liver						
Centrilobular pallor	0	3	8**	9**	8**	10**
Pancreatic acinar cell						
Degeneration	0	0	9**	8**	7**	8**
Mesenteric lymph node						
Reticulum cell hyperplasia	2	9**	10**	10**	10**	9**
Female						
n	10	10	10	10	10	10
Liver						
Pigment	0	9**	10**	10**	10**	9**
Pancreatic acinar cell						
Degeneration	0	5*	9**	6**	9**	9**
Kidney						
Tubule regeneration	2	1	7*	5	7*	7*
Tubule pigment	0	0	2	5*	3	9*
Chronic inflammation	1	3	5	7**	6*	8**
Karyomegaly	0	0	1	9**	10**	10**
Mesenteric lymph node						
Reticulum cell hyperplasia	3	–[a]	2	10**	10**	9**

* Significantly different (P≤0.05) from the control group by Fisher exact test
** P≤0.01
[a] Tissue not examined at this dose level

(centrilobular pallor). Livers of most treated females contained brown pigment (presumably hemosiderin) within scattered Kupffer cells. Degeneration of scattered individual pancreatic acinar cells was observed in treated males and females and was characterized by the lack of cytoplasmic staining sometimes accompanied by individual cell necrosis. The incidence of reticulum cell hyperplasia of the mesenteric lymph node was increased in treated males and females. This change consisted of multiple small clusters of reticulum cells within the medullary portion of the node.

Tubule regeneration, characterized by slightly dilated tubules lined by small basophilic epithelial cells, and chronic inflammation, consisting of occasional clusters of lymphocytes within the cortical interstitium, occurred more frequently in treated females. These changes are characteristic of the nephropathy commonly observed in aging Fischer rats. In addition, minimal amounts of brownish pigment within tubule epithelial cells and enlargement of scattered tubule epithelial nuclei (karyomegaly) were observed in treated females.

Dose selection rationale: Based on decreases in body weight and in water consumption and organ toxicity, the doses selected for the 2-year studies were 0, 70, 150, and 300 ppm C.I. Acid Red 114 for male rats and 0, 150, 300, and 600 ppm for females.

2-YEAR STUDIES

9-Month Interim Evaluations

Necropsy body weights for females receiving 600 ppm C.I. Acid Red 114 were significantly lower ($P \leq 0.05$) than the controls (Table D3). In the high-dose males and females, absolute and relative liver weights were significantly increased ($P \leq 0.01$). In high-dose males, absolute kidney weight was greater than the controls. In high-dose females, both absolute and relative kidney weights were greater than the controls.

Hematology, clinical chemistry, and urinalysis results are presented in Table E2. Several urinalysis parameters in 300 ppm dosed males and 600 ppm dosed females were significantly increased. Those parameters increased included urine osmolality, osmolality ratio, urine creatinine, and specific gravity, while urine volume was significantly decreased. In dosed females, hematocrit, hemoglobin, and erythrocyte counts were significantly decreased, indicating the development of mild anemia as seen in the 13-week studies. Lactate dehydrogenase and sorbitol dehydrogenase levels were significantly increased, which is consistent with hepatocellular damage. Triiodothyronine and thyroxine levels were significantly decreased and may have been secondary to reduced production of thyroid-binding globulin by the liver.

At 9 months, a few neoplastic lesions were observed including one neoplastic nodule (hepatocellular adenoma) and one alveolar/bronchiolar adenoma in high-dose males, and one clitoral gland carcinoma in a high-dose female (Table 6). In addition, there were a few nonneoplastic changes in treated animals including cytoplasmic vacuolization, clear cell foci, and hepatocyte hypertrophy in the liver; an increase in the severity of nephropathy in the kidneys of high-dose males; and an increase in the incidence and severity of nephropathy in high-dose females.

TABLE 6
Incidences of Selected Treatment-Related Lesions in Rats at the 9-Month Interim Evaluations in the 2-Year Drinking Water Studies of C.I. Acid Red 114

	Male		Female	
	0 ppm	300 ppm	0 ppm	600 ppm
n	10	10	10	10
Liver				
Neoplastic nodule[a]	0	1	0	0
Vacuolization, cytoplasmic	0	10** (1.0)[b]	0	2 (1.5)
Basophilic focus	0	10** (1.4)	0	10** (1.0)
Clear cell focus	0	7** (1.0)	0	0
Hepatocyte hypertrophy	0	0	0	10** (2.0)
Lung				
Alveolar/bronchiolar adenoma	0	1	0	0
Clitoral gland				
Carcinoma	–	–	0	1
Kidney				
Nephropathy	10 (1.1)	10 (1.5)	3 (1.0)	10** (1.5)

** Significantly different ($P \leq 0.01$) from the control group by Fisher exact test
[a] Term previously used for neoplasm now diagnosed as hepatocellular adenoma.
[b] Values in parentheses are average severity grades of lesions in affected animals; 1 = minimal and 2 = mild.

15-Month Interim Evaluations

Absolute and relative liver weights were significantly greater ($P \leq 0.01$) than the controls for males receiving 300 ppm and for females in all dosed groups (Table D4). Relative kidney weights were increased in mid- and high-dose females.

Hematology, clinical chemistry, and urinalysis results are presented in Table E3. Several hematology parameters, including hematocrit, hemoglobin, erythrocyte count, and mean cell volume, were decreased in 300 ppm dosed males and 600 ppm dosed females. These findings were consistent with a poorly regenerative anemia. Segmented neutrophils were significantly increased in the 300 and 600 ppm dosed females, presumably as a result of inflammation associated with neoplasms occurring in these groups. Alanine aminotransferase was significantly increased in 600 ppm dosed females and sorbitol dehydrogenase was significantly increased in all dosed females and is indicative of hepatocellular damage.

Triiodothyronine and thyroid-stimulating hormone (TSH) levels were significantly increased in 600 ppm dosed females; increased TSH levels are consistent with the marginal increase in proliferative thyroid follicular cell lesions seen in the 2-year studies. Urine osmolality, osmolality ratio, urine creatinine, and specific gravity were significantly increased in 300 ppm dosed males, while urine volume was significantly decreased, and these effects are considered to be secondary to decreased water intake.

A variety of lesions were found in male and female rats given C.I. Acid Red 114 in drinking water for 15 months (Table 7). Proliferative lesions included hepatocellular adenoma (neoplastic nodule) and hepatocellular carcinoma in the liver; alveolar/bronchiolar adenoma and alveolar epithelial hyperplasia of the lung; sebaceous gland adenoma and squamous cell carcinoma of the skin; Zymbal's gland adenoma, carcinoma, and hyperplasia; adenoma, carcinoma, and hyperplasia of the clitoral gland; squamous cell papilloma and carcinoma in the oral cavity epithelium (palate and tongue); and adenocarcinoma of the small and large intestine. Additional treatment-related nonneoplastic effects included cytoplasmic vacuolization, cystic degeneration, hepatocyte hypertrophy, and clear and basophilic foci in the liver; an increase in the severity of nephropathy in treated males; and an increase in the incidence and severity of nephropathy in treated females.

TABLE 7
Incidences of Selected Treatment-Related Lesions in Rats at the 15-Month Interim Evaluations in the 2-Year Drinking Water Studies of C.I. Acid Red 114

Male	0 ppm	70 ppm	150 ppm	300 ppm
n	10	10	10	10
Liver				
Neoplastic nodule[a]	0	0	0	1
Vacuolization, cytoplasmic	1	6* (1.2)	5 (1.2)	10** (2.2)
Cystic degeneration	0	1	3 (1.0)	3 (1.3)
Basophilic focus	3 (1.0)[b]	8* (1.1)	10** (1.0)	10** (2.3)
Clear cell focus	1	2 (1.5)	2 (2.0)	7** (1.4)
Hepatocyte hypertrophy	0	0	1	2 (1.0)
Lung				
Alveolar/bronchiolar adenoma	0	1	0	0
Hyperplasia, alveolar epithelium	1	2 (1.5)	3 (2.0)	4 (2.3)
Skin				
Sebaceous gland adenoma	0	0	0	1
Squamous cell carcinoma	0	0	0	1
Tongue				
Squamous cell carcinoma	0	0	1	0
Zymbal's gland				
Adenoma	0	0	1	1
Kidney				
Nephropathy	10 (1.5)	10 (1.7)	10 (2.1)	10 (2.5)

Female	0 ppm	150 ppm	300 ppm	600 ppm
n	10	10	10	10
Liver				
Hepatocellular carcinoma	0	0	0	5*
Neoplastic nodule[a]	0	2	1	6**
Vacuolization, cytoplasmic	2 (1.0)	3 (1.7)	9** (1.6)	9** (3.1)
Cystic degeneration	0	0	2 (1.5)	2 (1.5)
Basophilic focus	6 (1.0)	9 (1.3)	10* (1.9)	10* (1.4)
Hepatocyte hypertrophy	0	4* (1.0)	9** (1.8)	10** (3.6)
Lung				
Alveolar/bronchiolar adenoma	0	0	0	3
Hyperplasia, alveolar epithelium	0	2 (1.0)	3 (1.0)	8** (2.3)

TABLE 7
Incidences of Selected Treatment-Related Lesions in Rats at the 15-Month Interim Evaluations in the 2-Year Drinking Water Studies of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Female (continued)				
n	10	10	10	10
Clitoral gland				
Adenoma	0	1	3	4*
Carcinoma	0	0	0	2
Hyperplasia, squamous	0	0	2 (2.0)	2 (3.0)
Oral cavity (palate and tongue)				
Squamous cell papilloma	0	0	0	4*
Skin				
Squamous cell carcinoma	0	0	1	0
Small intestine				
Adenocarcinoma	0	0	1	1
Large intestine				
Adenocarcinoma	0	0	0	1
Zymbal's gland				
Papilloma	0	0	0	2
Carcinoma	0	0	1	1
Hyperplasia, squamous	0	2 (2.0)	2 (1.5)	6** (1.7)
Kidney				
Nephropathy	7 (1.1)	9 (1.1)	10 (2.0)	10 (3.9)

* Significantly different ($P \leq 0.05$) from the control group by Fisher exact test
** $P \leq 0.01$
[a] Term previously used for neoplasm now diagnosed as hepatocellular adenoma.
[b] Values in parentheses are average severity grades of lesions in affected animals; 1 = minimal; 2 = mild, 3 = moderate, and 4 = marked.

Body Weights, Water Consumption, and Clinical Findings in the 2-Year Studies

At week 105 of the 2-year studies, final mean body weights relative to controls for male rats in 70 ppm, 150 ppm, and 300 ppm dose groups were 94%, 90%, and 90%, and for females in 150 ppm and 300 ppm dose groups, relative weights were 99% and 89% (Tables 8 and 9, Figure 2). These body weight decrements began in the second year of the studies and were considered to be due to the development of neoplastic disease in dosed animals. Female rats in the 600 ppm dose group only survived until week 88; their final mean body weight relative to controls was 72%.

Mean water consumption was, in general, similar (±10%) among the dosed and control groups, except in 300 ppm dosed males and 600 ppm dosed females where water consumption was increased during the last year of the studies. This increase was attributed, in part, to an increase in the severity of nephropathy relative to the controls.

The daily dose of C.I. Acid Red 114 during weeks 53 to 101 was approximately 4, 8, and 20 mg/kg for males receiving 70, 150, and 300 ppm; and 9, 21, and 69 mg/kg for females receiving 150, 300, and 600 ppm (Tables G1 and G2). Tissue masses and swellings were the most common clinical findings in dosed rats. Red staining of the fur was noted in all dosed groups.

TABLE 8
Mean Body Weights and Survival of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	70 ppm Av. Wt. (g)	70 ppm Wt. (% of controls)	70 ppm No. of Survivors	150 ppm Av. Wt. (g)	150 ppm Wt. (% of controls)	150 ppm No. of Survivors	300 ppm Av. Wt. (g)	300 ppm Wt. (% of controls)	300 ppm No. of Survivors
1	118	70	119	101	45	117	100	75	119	101	70
2	159	70	160	100	45	155	98	75	156	98	70
3	199	70	196	99	45	196	99	75	195	98	70
4	227	70	228	100	45	230	101	75	227	100	70
5	249	70	255	102	45	251	101	75	253	101	70
6	272	70	271	100	45	274	101	75	271	100	70
7	287	70	287	100	45	286	100	75	280	98	70
8	300	70	302	101	45	305	102	75	297	99	70
9	314	70	314	100	45	319	101	75	315	100	70
10	331	70	330	100	45	334	101	75	329	100	70
11	332	70	342	103	45	344	104	75	339	102	70
12	346	70	349	101	45	354	102	75	348	101	70
13	352	70	347	98	45	358	102	75	352	100	70
14	360	70	359	100	45	364	101	75	358	100	70
15	361	70	371	103	45	371	103	75	368	102	70
17	370	70	377	102	45	382	103	75	378	102	70
21	396	69	391	99	45	392	99	75	387	98	70
25	418	69	415	99	45	422	101	75	421	101	70
29	423	69	423	100	45	432	102	75	428	101	70
33	436	69	438	101	45	437	100	75	435	100	70
37	443	69	442	100	45	449	101	75	443	100	70
41	449	59[a]	455	101	45	452	101	75	450	100	60[a]
45	457	59	455	100	45	457	100	75	455	100	59
49	462	59	459	99	45	463	100	74	458	99	59
53	460	58	463	101	44	466	101	74	456	99	59
57	454	58	453	100	44	453	100	74	445	98	57
61	454	58	459	101	44	462	102	73	451	99	56
65	459	58	460	100	43	463	101	73	452	99	56
69[a]	458	45	452	99	32	455	100	62	442	97	43
73	459	45	450	98	32	451	98	61	435	95	42
77	457	44	452	99	29	452	99	60	436	95	32
81	450	44	447	99	28	445	99	60	427	95	29
85	445	42	448	101	28	443	100	59	411	92	27
89	455	39	444	98	28	437	96	54	400	88	26
93	445	37	427	96	26	421	95	48	388	87	20
95	449	35	426	95	22	413	92	45	365	81	17
97	439	34	422	96	21	407	93	41	359	82	12
99	440	32	413	94	21	401	91	38	365	83	11
101	434	30	417	96	18	410	95	30	352	81	6
103	446	25	419	94	16	406	91	26	356	80	3
105	437	24	411	94	15	393	90	26	392	90	1
Terminal sacrifice		24			15			26			1
Mean for weeks											
1-13	268		269	100		271	101		268	100	
14-52	416		417	100		420	101		416	100	
53-105	449		439	98		434	97		408	91	

[a] Interim evaluation occurred.

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	150 ppm Av. Wt. (g)	150 ppm Wt. (% of controls)	150 ppm No. of Survivors	300 ppm Av. Wt. (g)	300 ppm Wt. (% of controls)	300 ppm No. of Survivors	600 ppm Av. Wt. (g)	600 ppm Wt. (% of controls)	600 ppm No. of Survivors
1	96	70	96	100	45	97	101	75	98	102	70
2	119	70	119	99	45	116	97	75	117	98	70
3	137	70	136	99	45	133	97	75	133	97	70
4	150	70	148	99	45	146	97	75	145	97	70
5	159	70	162	102	45	155	98	75	156	98	70
6	170	70	168	99	45	163	96	75	161	95	70
7	177	70	175	99	45	170	96	75	168	95	70
8	183	70	184	100	45	176	96	75	174	95	70
9	187	70	186	100	45	180	97	74	181	97	70
10	194	70	193	99	45	187	96	74	184	95	70
11	196	70	197	101	45	190	97	74	186	95	70
12	201	70	204	101	45	194	96	74	191	95	70
13	204	70	203	100	45	196	96	74	193	95	70
14	206	70	208	101	45	199	97	73	196	95	70
15	209	70	210	100	45	203	97	73	200	96	70
17	216	70	214	99	45	210	97	73	208	96	70
21	219	70	220	100	45	211	97	73	209	96	70
25	231	70	232	101	45	224	97	73	221	96	70
29	235	70	233	99	45	229	97	73	223	95	69
33	242	70	244	101	45	231	96	73	228	94	69
37	247	70	248	100	45	239	97	73	233	94	68
41	255	60[a]	259	102	45	244	96	73	237	93	67
45	263	60	269	102	45	252	96	72	242	92	56
49	272	60	274	101	45	260	96	72	242	89	56
53	286	60	290	101	45	267	93	71	249	87	50
57	290	60	293	101	45	278	96	71	250	86	44
61	300	60	305	102	43	287	96	69	250	83	33
65	307	59	312	102	43	292	95	68	254	83	29
69[a]	320	49	315	98	32	296	93	57	252	79	14
73	330	48	323	98	31	300	91	52	271	82	8
77	334	47	332	99	30	309	93	45	258	77	8
81	344	45	335	97	29	307	90	41	270	79	5
85	345	42	341	99	27	306	89	34	249	72	3
89	352	42	341	97	25	306	87	27			
93	352	41	346	98	22	300	85	21			
95	354	41	341	96	20	295	84	17			
97	353	40	345	98	17	288	82	17			
99	357	39	348	98	16	294	83	14			
101	358	39	349	98	16	298	83	11			
103	360	36	345	96	14	299	83	6			
105	354	36	350	99	13	296	84	6			
Terminal sacrifice		36			13			6			0
Mean for weeks											
1-13	167		167	100		162	97		161	96	
14-52	236		237	100		227	96		222	94	
53-105	335		330	99		295	88		256	76	

[a] Interim evaluation occurred.

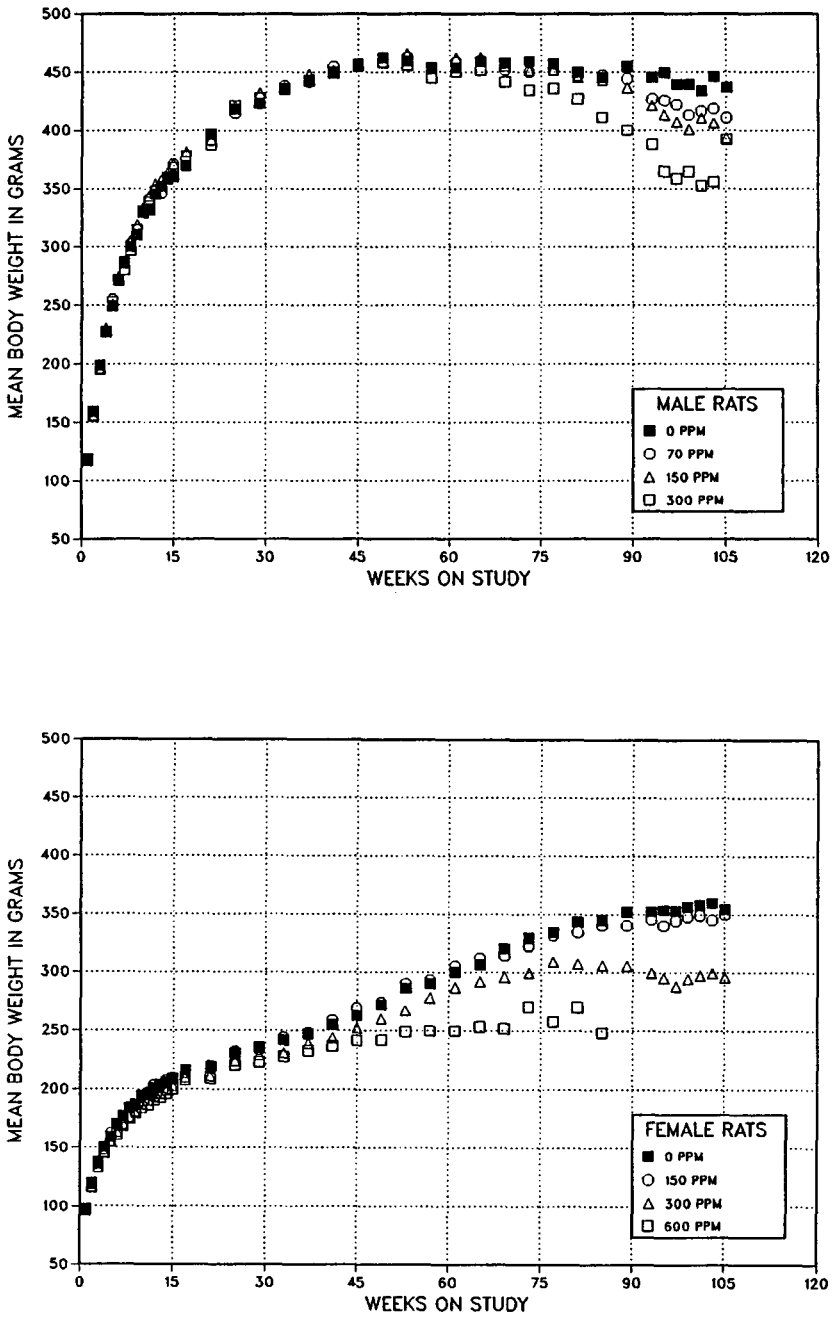


Figure 2
Growth Curves for Male and Female Rats Administered C.I. Acid Red 114 in Drinking Water for 2 Years

Survival

Estimates of the probabilities of survival for male and female rats given C.I. Acid Red 114 and the controls are shown in Table 10 and in the Kaplan-Meier survival curves in Figure 3. Decreases in survival in females receiving 600 ppm began after week 52 and all had died by week 88. Final survival was decreased in males receiving 70 ppm and in females receiving 150 ppm. These decreases in survival were due to the development of neoplasms in dosed animals.

TABLE 10
Survival of Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114

Male	0 ppm	70 ppm	150 ppm	300 ppm
Animals initially in study	70	45	75	70
Natural deaths	13	11	16	16
Moribund	13	9	23	33
Interim evaluations[a]				
9 month	10	0	0	10
15 month	10	10	10	10
Animals surviving to study termination	24[b]	15	26	1
Percent survival at end of study[c]	49	43	40	2
Mean survival days[d]	579	616	643	527
Survival analysis[e]	P<0.001	P=0.730	P=0.507	P<0.001

Female	0 ppm	150 ppm	300 ppm	600 ppm
Animals initially in study	70	45	75	70
Natural deaths	4	6	17	13
Moribund	10	16	41	37
Accidental deaths[a]	0	0	1	0
Interim evaluations[a]				
9 month	10	0	0	10
15 month	10	10	10	10
Animals surviving to study termination	36	13	6	0
Percent survival at end of study[c]	72	38	10	0
Mean survival days[d]	602	609	562	412
Survival analysis[e]	P<0.001	P=0.002	P<0.001	P<0.001

[a] Censored from survival analyses.
[b] One of these animals was dead on the last day of the studies.
[c] Kaplan-Meier determinations. Survival rates adjusted for accidental deaths and interim evaluations.
[d] Mean of all deaths (uncensored, censored, terminal sacrifice).
[e] The entry under the "0 ppm" column is associated with the life table trend test (Tarone, 1975). Subsequent entries are the results of pairwise tests (Cox, 1972).

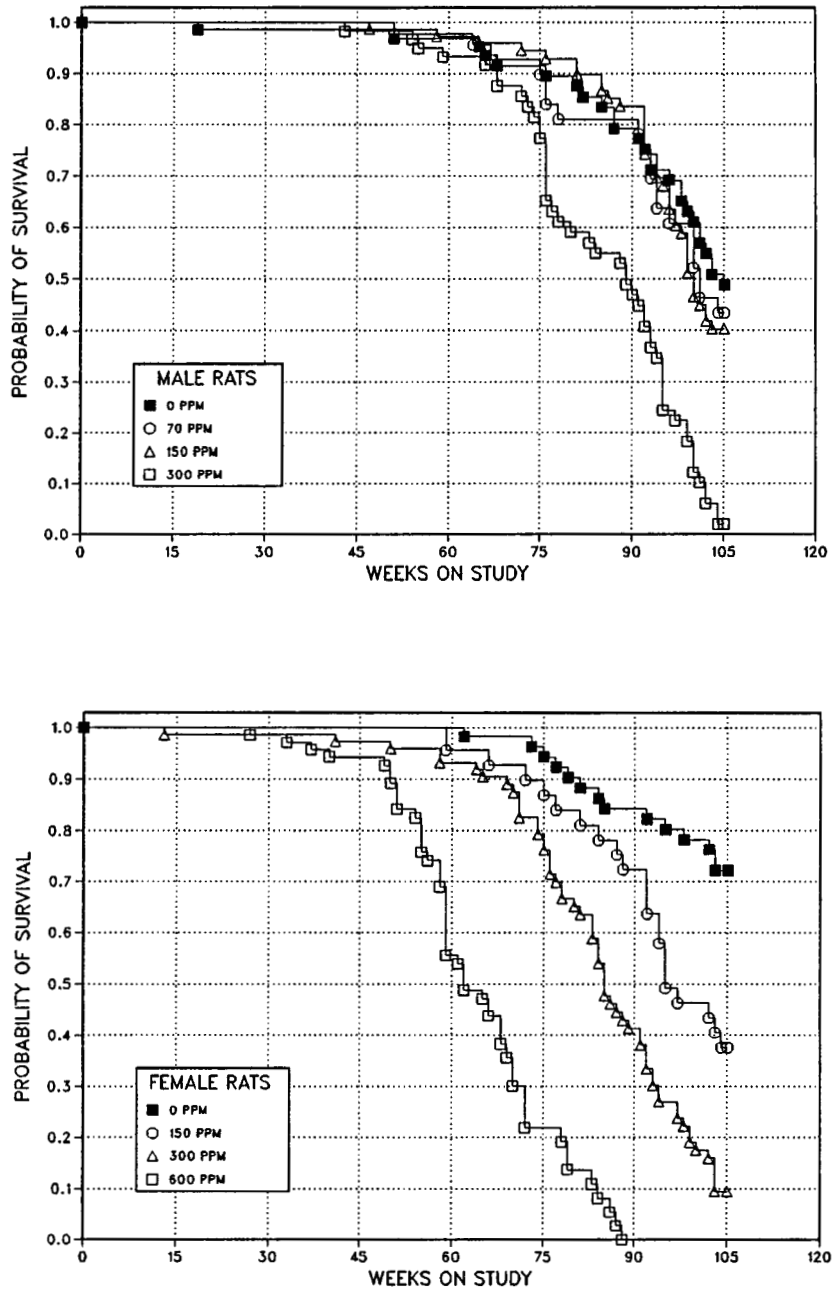


Figure 3
Kaplan-Meier Survival Curves for Male and Female Rats Administered C.I. Acid Red 114 in Drinking Water for 2 Years

Pathology and Statistical Analyses of Results

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

Skin: A variety of epithelial neoplasms of the skin occurred with increased incidences in male and female rats treated with C.I. Acid Red 114 (Table 11). The incidences of basal cell adenomas, basal cell carcinomas, and the combined incidence of basal cell adenomas and carcinomas were moderately increased in low-dose (70 ppm) males and significantly increased in mid- (150 ppm) and high-dose (300 ppm) males. Many of the treated males had multiple basal cell adenomas. There was a statistically significant increase in the incidence of basal cell neoplasms in females from the low- (150 ppm) and mid-dose (300 ppm) groups. The incidence of sebaceous gland adenomas was increased in treated males and was significantly increased in the high-dose group. The combined incidences of squamous cell papilloma and squamous cell carcinoma were significantly increased in mid- and high-dose males, and in mid-dose females. The lower incidence of skin neoplasms in the high-dose (600 ppm) female group was considered secondary to reduced survival in this group due to neoplasms at other sites. The incidence of keratoacanthomas was increased in dosed male groups compared to controls and was significantly increased in the high-dose group. A single keratoacanthoma occurred in one high-dose female.

Basal cell neoplasms were composed of small basophilic polygonal cells that formed sheets, tortuous cords, or solid lobules that often contained central cavities. Adenomas were discrete, well demarcated masses while carcinomas exhibited local invasion and frequently contained areas of necrosis. Many basal cell neoplasms contained areas of squamous, sebaceous, or hair follicle differentiation (Plate 1). Some neoplasms consisted solely of sebaceous elements and were diagnosed as sebaceous gland adenoma or carcinoma. Squamous cell papillomas were exophytic growths that were pedunculated and highly branched, and composed of a fibrovascular core covered by thickened, stratified, squamous epithelium. Squamous cell carcinomas were highly invasive lesions consisting of multiple irregular cords of disordered, pleomorphic, stratified, squamous epithelial cells, often containing foci of keratin formation, which projected into the dermis (Plate 2). Keratoacanthomas were cystic structures lying within the dermis that were lined by a thick, highly folded layer of heavily keratinized, stratified, squamous epithelium.

Zymbal's Gland: The Zymbal's gland is a specialized sebaceous gland that lies ventral and anterior to the orifice of the external ear. Zymbal's gland neoplasms grow very rapidly and generally lead to death of the animal; therefore, the life table analysis is the most appropriate statistical test for the incidences of these neoplasms. There were moderate increases in the incidences of Zymbal's gland neoplasms in treated males and marked increases in treated female rats (Table 12). The combined incidences of adenomas and carcinomas were significantly increased in the mid- and high-dose male groups and in all groups of treated females. Zymbal's glands from some treated animals contained nonneoplastic lesions including focal hyperplasia of the squamous epithelial lining of glandular ducts (hyperplasia, squamous) and dilatation of glandular ducts (ectasia)(Tables A5 and B5). There was a morphologic continuum from adenoma to carcinoma. Adenomas were discrete nodular masses composed of glandular acini of relatively normal appearing sebaceous cells and containing ductular structures lined by stratified squamous epithelium. Occasionally, these ductular structures were dilated and filled with secretory material. Carcinomas were generally larger and invaded adjacent tissues. Neoplastic cells were often atypical. They exhibited disordered growth patterns and formed solid masses, irregular acinar structures, and cords, with a scattering of ductular structures. Occasionally, areas of necrosis were observed. Some carcinomas consisted principally of sebaceous cells while others were composed mainly of stratified squamous epithelium; some neoplasms had prominent components of both.

TABLE 11
Skin Neoplasms in Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114

Male	0 ppm	70 ppm	150 ppm	300 ppm
Skin: Keratoacanthoma[a]				
Overall rates[b]	1/50 (2%)	1/35 (3%)	4/65 (6%)	7/50 (14%)
Effective rates[c]	1/42 (2%)	1/28 (4%)	4/58 (7%)	7/28 (25%)
Terminal rates[d]	1/24 (4%)	1/15 (7%)	4/26 (15%)	1/1 (100%)
First incidence (days)	732 (T)	732 (T)	732 (T)	582
Life table tests[e]	P<0.001	P=0.654	P=0.200	P<0.001
Logistic regression tests[e]	P<0.001	P=0.654	P=0.200	P=0.005
Skin (Sebaceous Gland): Adenoma or Carcinoma[f]				
Overall rates	1/50 (2%)	1/35 (3%)	5/65 (8%)	6/50 (12%)
Effective rates	1/45 (2%)	1/32 (3%)	5/61 (8%)	6/41 (15%)
Terminal rates	0/24 (0%)	1/15 (7%)	4/26 (15%)	0/1 (0%)
First incidence (days)	706	732 (T)	593	512
Life table tests	P<0.001	P=0.638	P=0.132	P=0.001
Logistic regression tests	P=0.007	P=0.663	P=0.166	P=0.036
Skin: Basal Cell Adenoma				
Overall rates	1/50 (2%)	4/35 (11%)	26/65 (40%)	30/50 (60%)
Effective rates	1/46 (2%)	4/32 (13%)	26/62 (42%)	30/44 (68%)
Terminal rates	1/24 (4%)	3/15 (20%)	19/26 (73%)	1/1 (100%)
First incidence (days)	732 (T)	651	641	473
Life table tests	P<0.001	P=0.071	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.073	P<0.001	P<0.001
Skin: Basal Cell Carcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	5/65 (8%)	6/50 (12%)
Effective rates	0/46 (0%)	1/32 (3%)	5/62 (8%)	6/44 (14%)
Terminal rates	0/24 (0%)	0/15 (0%)	4/26 (15%)	0/1 (0%)
First incidence (days)	–[g]	724	673	473
Life table tests	P<0.001	P=0.411	P=0.043	P=0.002
Logistic regression tests	P=0.003	P=0.408	P=0.046	P=0.020
Skin: Basal Cell Adenoma or Carcinoma[h]				
Overall rates	1/50 (2%)	5/35 (14%)	28/65 (43%)	32/50 (64%)
Effective rates	1/46 (2%)	5/32 (16%)	28/62 (45%)	32/44 (73%)
Terminal rates	1/24 (4%)	3/15 (20%)	20/26 (77%)	1/1 (100%)
First incidence (days)	732 (T)	651	641	473
Life table tests	P<0.001	P=0.032	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.030	P<0.001	P<0.001
Skin: Squamous Cell Papilloma				
Overall rates	1/50 (2%)	0/35 (0%)	3/65 (5%)	2/50 (4%)
Effective rates	1/35 (3%)	0/23 (0%)	3/48 (6%)	2/18 (11%)
Terminal rates	1/24 (4%)	0/15 (0%)	1/26 (4%)	0/1 (0%)
First incidence (days)	732 (T)	–	654	704
Life table tests	P=0.020	P=0.594N	P=0.368	P=0.012
Logistic regression tests	P=0.093	P=0.594N	P=0.401	P=0.078
Skin: Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	2/35 (6%)	8/65 (12%)	7/50 (14%)
Effective rates	0/45 (0%)	2/32 (6%)	8/61 (13%)	7/41 (17%)
Terminal rates	0/24 (0%)	2/15 (13%)	5/26 (19%)	0/1 (0%)
First incidence (days)	–	732 (T)	565	512
Life table tests	P<0.001	P=0.141	P=0.009	P=0.002
Logistic regression tests	P=0.006	P=0.141	P=0.013	P=0.017

TABLE 11
Skin Neoplasms in Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114
(continued)

Male (continued)	0 ppm	70 ppm	150 ppm	300 ppm
Skin: Squamous Cell Papilloma or Squamous Cell Carcinoma[i]				
Overall rates	1/50 (2%)	2/35 (6%)	11/65 (17%)	9/50 (18%)
Effective rates	1/45 (2%)	2/32 (6%)	11/61 (18%)	9/41 (22%)
Terminal rates	1/24 (4%)	2/15 (13%)	6/26 (23%)	0/1 (0%)
First incidence (days)	732 (T)	732 (T)	565	512
Life table tests	P<0.001	P=0.336	P=0.007	P<0.001
Logistic regression tests	P=0.001	P=0.336	P=0.010	P=0.011

Female	0 ppm	150 ppm	300 ppm	600 ppm
Skin: Keratoacanthoma[j]				
Overall rates	0/50 (0%)	0/35 (0%)	0/65 (0%)	1/50 (2%)
Skin: Basal Cell Adenoma				
Overall rates	0/50 (0%)	3/35 (9%)	5/65 (8%)	3/50 (6%)
Effective rates	0/49 (0%)	3/33 (9%)	5/58 (9%)	3/19 (16%)
Terminal rates	0/36 (0%)	2/13 (15%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	663	521	454
Life table tests	P<0.001	P=0.016	P=0.005	P=0.006
Logistic regression tests	P=0.040	P=0.036	P=0.049	P=0.211
Skin: Basal Cell Carcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	2/65 (3%)	2/50 (4%)
Skin: Basal Cell Adenoma or Carcinoma[k]				
Overall rates	0/50 (0%)	4/35 (11%)	7/65 (11%)	5/50 (10%)
Effective rates	0/50 (0%)	4/35 (11%)	7/62 (11%)	5/45 (11%)
Terminal rates	0/36 (0%)	2/13 (15%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	614	521	344
Life table tests	P<0.001	P=0.006	P<0.001	P<0.001
Logistic regression tests	P=0.012	P=0.020	P=0.013	P=0.071
Skin: Squamous Cell Papilloma				
Overall rates	0/50 (0%)	0/35 (0%)	1/65 (2%)	1/50 (2%)
Skin: Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	3/65 (5%)	0/50 (0%)
Effective rates	0/38 (0%)	0/15 (0%)	3/10 (30%)	0/0 (0%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	–	715	–
Life table tests	P=0.003	–	P=0.002	–
Logistic regression tests	P=0.009	–	P=0.007	–
Skin: Squamous Cell Papilloma or Squamous Cell Carcinoma[l]				
Overall rates	0/50 (0%)	0/35 (0%)	4/65 (6%)	1/50 (2%)
Effective rates	0/50 (0%)	0/35 (0%)	4/60 (7%)	1/33 (3%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	–	614	403
Life table tests	P<0.001	–	P=0.001	P=0.417
Logistic regression tests	P=0.034	–	P=0.011	P=0.931

TABLE 11
Skin Neoplasms in Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114
(continued)

(T)Terminal sacrifice
[a] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 34/681 (5.0% ± 3.0%); range 2%-11%
[b] Number of lesion-bearing animals/number of animals necropsied or examined microscopically for this tumor type
[c] Number of lesion-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this tumor type in any of the groups
[d] Observed incidence at terminal kill
[e] Beneath the "0 ppm" column are the P values associated with the trend test. Beneath the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. For all tests, a negative trend or a lower incidence in a dosed group is indicated by N.
[f] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 3/681 (0.4% ± 0.9%); range 0%-2%
[g] Not applicable; no tumors in animal group
[h] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 6/681 (0.9% ± 1.3%); range 0%-6%
[i] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 17/681 (2.5% ± 1.5%); range 0%-4%
[j] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 4/680 (0.6% ± 1.0%); range 0%-2%
[k] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 3/680 (0.4% ± 0.7%); range 0%-2%
[l] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 5/680 (0.7% ± 0.8%); range 0%-2%

TABLE 12
Zymbal's Gland Neoplasms in F344/N Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114

Male	0 ppm	70 ppm	150 ppm	300 ppm
Zymbal's Gland: Adenoma				
Overall rates[a]	0/50 (0%)	0/35 (0%)	1/65 (2%)	1/50 (2%)
Zymbal's Gland: Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	7/65 (11%)	6/50 (12%)
Effective rates[b]	0/49 (0%)	0/35 (0%)	7/65 (11%)	6/49 (12%)
Terminal rates[c]	0/24 (0%)	0/15 (0%)	2/26 (8%)	0/1 (0%)
First incidence (days)	–[d]	–	325	524
Life table tests[e]	P<0.001	–	P=0.022	P=0.002
Logistic regression tests[e]	P=0.011	–	P=0.013	P=0.018
Zymbal's Gland: Adenoma or Carcinoma[f]				
Overall rates	0/50 (0%)	0/35 (0%)	8/65 (12%)	7/50 (14%)
Effective rates	0/49 (0%)	0/35 (0%)	8/65 (12%)	7/49 (14%)
Terminal rates	0/24 (0%)	0/15 (0%)	2/26 (8%)	0/1 (0%)
First incidence (days)	–	–	325	524
Life table tests	P<0.001	–	P=0.014	P<0.001
Logistic regression tests	P=0.005	–	P=0.008	P=0.009

Female	0 ppm	150 ppm	300 ppm	600 ppm
Zymbal's Gland: Adenoma				
Overall rates	0/50 (0%)	0/35 (0%)	2/65 (3%)	6/50 (12%)
Effective rates	0/50 (0%)	0/33 (0%)	2/59 (3%)	6/27 (22%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	–	599	412
Life table tests	P<0.001	–	P=0.060	P<0.001
Logistic regression tests	P<0.001	–	P=0.191	P=0.007
Zymbal's Gland: Carcinoma				
Overall rates	0/50 (0%)	3/35 (9%)	17/65 (26%)	13/50 (26%)
Effective rates	0/50 (0%)	3/35 (9%)	17/61 (28%)	13/42 (31%)
Terminal rates	0/36 (0%)	1/13 (8%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	411	403	355
Life table tests	P<0.001	P=0.042	P<0.001	P<0.001
Logistic regression tests	P=0.010	P=0.163	P<0.001	P=0.021
Zymbal's Gland: Adenoma or Carcinoma[g]				
Overall rates	0/50 (0%)	3/35 (9%)	18/65 (28%)	19/50 (38%)
Effective rates	0/50 (0%)	3/35 (9%)	18/61 (30%)	19/42 (45%)
Terminal rates	0/36 (0%)	1/13 (8%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	411	403	355
Life table tests	P<0.001	P=0.042	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.163	P<0.001	P<0.001

[a] Number of lesion-bearing animals/number of animals necropsied or examined microscopically for this tumor type
[b] Number of lesion-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this tumor type in any of the groups
[c] Observed incidence at terminal kill
[d] Not applicable; no tumors in animal group
[e] Beneath the "0 ppm" column are the P values associated with the trend test. Beneath the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal.
[f] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 11/681 (1.6% ± 1.7%); range 0%-4%
[g] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 6/680 (0.9% ± 1.0%); range 0%-2%

Clitoral Glands: The clitoral glands of the female rat are bilateral, modified sebaceous glands located near the base of the clitoris. There was a marked treatment-related increase in the incidence of clitoral gland neoplasms in female rats (Table 13). The combined incidence of adenomas and carcinomas was significantly increased in all treated female groups. A few treated females had bilateral adenomas or carcinomas. Focal glandular cell hyperplasia of the clitoral gland occurred at a slightly increased incidence in the mid-dose female group. Adenomas were discrete, well-demarcated, expansile masses displaying some loss of the normal acinar architecture. They were composed of relatively well-differentiated cells arranged in solid clusters; a few ductlike structures, sometimes containing debris, were scattered within the neoplasms. Carcinomas were poorly demarcated masses that sometimes invaded adjacent tissues (Plate 3). They were composed of solid sheets and clusters of disorganized pleomorphic cells, and often there was an abundance of small basophilic basal-like cells (reserve cells). Some carcinomas exhibited marked cellular atypia or contained large areas of necrosis.

TABLE 13
Clitoral Gland Proliferative Lesions in Female F344/N Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

	0 ppm	150 ppm	300 ppm	600 ppm
Clitoral Gland: Hyperplasia, glandular	4/48 (8%)	2/32 (6%)	8/62 (13%)	2/50 (4%)
Clitoral Gland: Adenoma				
Overall rates[a]	7/48 (15%)	10/32 (31%)	10/62 (16%)	10/50 (20%)
Effective rates[b]	7/48 (15%)	10/32 (31%)	10/59 (17%)	10/43 (23%)
Terminal rates[c]	5/34 (15%)	4/11 (36%)	3/6 (50%)	0/0 (0%)
First incidence (days)	663	538	403	351
Life table tests[d]	P<0.001	P=0.004	P=0.002	P<0.001
Logistic regression tests[d]	P=0.091	P=0.028	P=0.271	P=0.231
Clitoral Gland: Carcinoma				
Overall rates	4/48 (8%)	9/32 (28%)	19/62 (31%)	15/50 (30%)
Effective rates	4/48 (8%)	9/32 (28%)	19/60 (32%)	15/46 (33%)
Terminal rates	2/34 (6%)	4/11 (36%)	3/6 (50%)	0/0 (0%)
First incidence (days)	641	411	400	339
Life table tests	P<0.001	P=0.003	P<0.001	P<0.001
Logistic regression tests	P=0.003	P=0.030	P=0.001	P=0.022
Clitoral Gland: Adenoma or Carcinoma[e]				
Overall rates	11/48 (23%)	17/32 (53%)	28/62 (45%)	23/50 (46%)
Effective rates	11/48 (23%)	17/32 (53%)	28/60 (47%)	23/46 (50%)
Terminal rates	7/34 (21%)	8/11 (73%)	6/6 (100%)	0/0 (0%)
First incidence (days)	641	411	400	339
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.003	P=0.001	P=0.014

[a] Number of lesion-bearing animals/number of animals examined microscopically for this tumor type
[b] Number of lesion-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this tumor type in any of the groups
[c] Observed incidence at terminal kill
[d] Beneath the "0 ppm" column are the P values associated with the trend test. Beneath the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal.
[e] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 74/606 (12.2% ± 5.7%); range 5%-23%

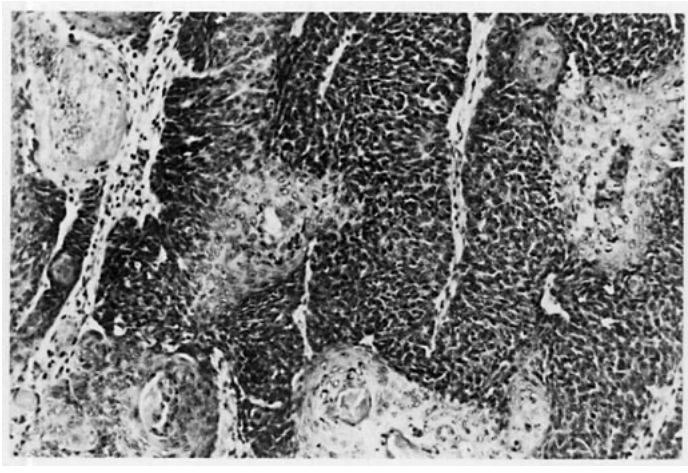


Plate 1
Basal cell carcinoma in the skin of a male rat from the 300 ppm dose group of the 2-year study. Broad cords of neoplastic basal cells have invaded the dermis. Areas of differentiation toward squamous epithelium and hair follicles are present. Magnification 120×

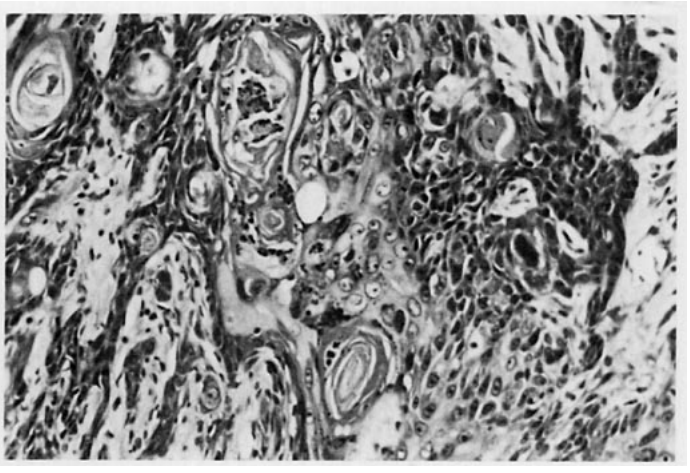


Plate 2
Squamous cell carcinoma in the skin of a male rat from the 300 ppm dose group of the 2-year study. Irregular cords and clusters of neoplastic stratified squamous epithelial cells with foci of keratinization have invaded the dermis. Magnification 190×

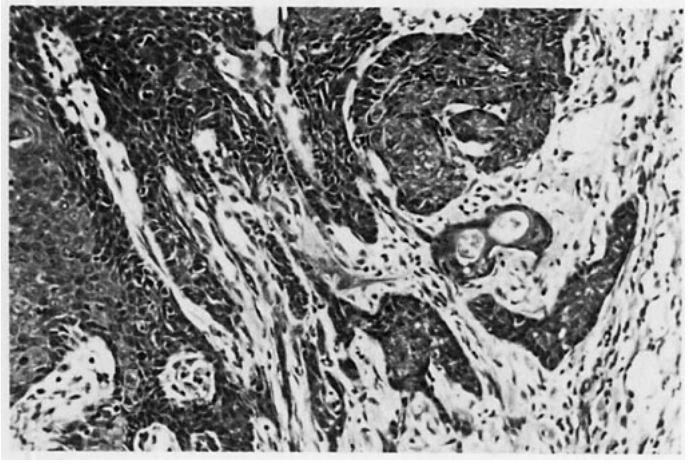


Plate 3
Clitoral gland carcinoma in a female rat from the 600 ppm dose group of the 2-year study. Irregular cords of glandular epithelial cells have invaded the adjacent connective tissue. Magnification 150×

Liver: The combined incidences of neoplastic nodules and hepatocellular carcinomas were significantly increased in mid- and high-dose male and female groups (Table 14). Livers of a few treated animals contained multiple neoplastic nodules. (Neoplastic nodule was the term previously used to refer to neoplasms currently diagnosed as hepatocellular adenomas.) These nodules were well-demarcated, expansile masses that compressed the adjacent parenchyma. Hepatic cords within neoplastic nodules were not organized in a normal lobular pattern and often intersected at near right angles with the cords of the adjacent normal liver cells. Neoplastic hepatocytes were slightly pleomorphic and exhibited increased eosinophilic staining. Hepatocellular carcinomas differed from neoplastic nodules in that neoplastic cells within carcinomas formed solid clusters, glandular structures, and broad trabeculae that were several (generally five or more) cell layers thick. Cells within carcinomas were often moderately to markedly pleomorphic and exhibited varying degrees of atypia.

A variety of nonneoplastic liver lesions, generally of minimal to mild severity, increased in incidence in treated males and females (Table 15). These lesions included eosinophilic foci, mixed cell foci, and hematopoietic cell proliferation, as well as degenerative changes characterized by cystic degeneration, hepatocyte necrosis, and fatty change. Eosinophilic foci consisted of clusters of hepatocytes with abundant, brightly eosinophilic cytoplasm. Foci caused little or no compression and blended smoothly with the surrounding parenchyma. Mixed cell foci were similar in appearance except that they consisted of a mixture of cells with either eosinophilic or clear cytoplasm. Cystic degeneration is a common degenerative lesion of the liver and is characterized by multiple focal clusters of variably sized cysts filled with granular eosinophilic materials or erythrocytes. The increase in hematopoietic cell proliferation was presumably secondary to inflammation associated with neoplasms in treated animals. Hepatocyte necrosis involved single or multiple small scattered foci of hepatocytes, which most commonly affected centrilobular hepatocytes. Fatty change ranged from focal to multifocal to diffuse and consisted of multiple, clear, discrete vacuoles within hepatocyte cytoplasm.

Lung: Alveolar/bronchiolar adenomas and carcinomas (combined) occurred with slightly increased incidences in dosed male rats (Table 16). The incidence in the high-dose male group was significantly increased. The incidence of alveolar epithelial hyperplasia was slightly increased in dosed males, but none of the increases were significant. The incidence of adenomas was significantly increased in the mid-dose female group, and the incidences of adenomas and carcinomas (combined) in the mid- and high-dose female groups exceeded the historical control range for untreated females from NTP 2-year studies (Table B4g). The incidence of focal or multifocal alveolar epithelial hyperplasia was numerically increased in mid- and high-dose males and females; the increase was significant in high-dose females. Alveolar/bronchiolar adenomas were discrete expansile masses that compressed adjacent lung parenchyma. They consisted of alveolar structures usually lined by a single layer of cuboidal to columnar cells. Carcinomas resembled adenomas, but cells within carcinomas showed more cellular atypia and tended to form multiple layers or solid sheets. Alveolar epithelial hyperplasia consisted of clusters of alveoli lined by a single layer of cuboidal cells; borders of hyperplastic areas tended to blend smoothly with the adjacent normal parenchyma.

TABLE 14
Liver Tumors in F344/N Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114

Male	0 ppm	70 ppm	150 ppm	300 ppm
Liver: Neoplastic Nodule				
Overall rates[a]	2/50 (4%)	1/35 (3%)	10/65 (15%)	15/50 (30%)
Effective rates[b]	2/44 (5%)	1/29 (3%)	10/60 (17%)	15/35 (43%)
Terminal rates[c]	1/24 (4%)	1/15 (7%)	7/26 (27%)	1/1 (100%)
First incidence (days)	607	732 (T)	641	531
Logistic regression tests[d]	P<0.001	P=0.631N	P=0.042	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	6/65 (9%)	7/50 (14%)
Effective rates	0/44 (0%)	1/31 (3%)	6/61 (10%)	7/38 (18%)
Terminal rates	0/24 (0%)	0/15 (0%)	5/26 (19%)	0/1 (0%)
First incidence (days)	–[e]	694	687	530
Logistic regression tests	P<0.001	P=0.423	P=0.023	P=0.008
Liver: Neoplastic Nodule or Hepatocellular Carcinoma[f]				
Overall rates	2/50 (4%)	2/35 (6%)	15/65 (23%)	20/50 (40%)
Effective rates	2/44 (5%)	2/31 (6%)	15/61 (25%)	20/38 (53%)
Terminal rates	1/24 (4%)	1/15 (7%)	11/26 (42%)	1/1 (100%)
First incidence (days)	607	694	641	530
Logistic regression tests	P<0.001	P=0.553	P=0.003	P<0.001

Female	0 ppm	150 ppm	300 ppm	600 ppm
Liver: Neoplastic Nodule				
Overall rates	0/50 (0%)	0/35 (0%)	15/64 (23%)	6/50 (12%)
Effective rates	0/50 (0%)	0/35 (0%)	15/60 (25%)	6/33 (18%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	–	403	411
Logistic regression tests	P<0.001	–	P<0.001	P=0.009
Liver: Hepatocellular Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	6/64 (9%)	3/50 (6%)
Effective rates	0/50 (0%)	0/35 (0%)	6/61 (10%)	3/34 (9%)
Terminal rates	0/36 (0%)	0/13 (0%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	–	400	501
Logistic regression tests	P=0.003	–	P=0.025	P=0.009
Liver: Neoplastic Nodule or Hepatocellular Carcinoma[g]				
Overall rates	0/50 (0%)	0/35 (0%)	19/64 (30%)	8/50 (16%)
Effective rates	0/50 (0%)	0/35 (0%)	19/61 (31%)	8/34 (24%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	–	400	411
Logistic regression tests	P<0.001	–	P<0.001	P<0.001

(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals necropsied or examined microscopically for this tumor type
[b] Number of lesion-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this tumor type in any of the groups
[c] Observed incidence at terminal kill
[d] Beneath the "0 ppm" column are the P values associated with the trend test. Beneath the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal. For all tests, a negative trend or a lower incidence in a dosed group is indicated by N.
[e] Not applicable; no tumors in animal group
[f] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 22/680 (3.2% ± 3.5%); range 0%-10%
[g] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 3/680 (0.4% ± 1.0%); range 0%-3%

TABLE 15
Nonneoplastic Liver Lesions in F344/N Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114

	0 ppm	70 ppm	150 ppm	300 ppm
Male				
Liver: Eosinophilic Focus				
Overall rates[a]	6/50 (12%)	5/35 (14%)	19/65 (29%)	26/50 (52%)
Adjusted rates[b]	23.1%	26.6%	54.7%	100.0%
Terminal rates[c]	5/24 (21%)	3/15 (20%)	12/26 (46%)	1/1 (100%)
First incidence (days)	671	641	641	473
Logistic regression tests[d]	P<0.001	P=0.461	P=0.013	P<0.001
Liver: Mixed Cell Focus				
Overall rates	7/50 (14%)	9/35 (26%)	24/65 (37%)	14/50 (28%)
Adjusted rates	29.2%	50.8%	68.1%	43.8%
Terminal rates	7/24 (29%)	7/15 (47%)	16/26 (62%)	0/1 (0%)
First incidence (days)	732 (T)	530	530	377
Logistic regression tests	P=0.012	P=0.097	P=0.003	P=0.065
Liver: Hematopoietic Cell Proliferation				
Overall rates	1/50 (2%)	2/35 (6%)	6/65 (9%)	14/50 (28%)
Adjusted rates	2.6%	8.0%	19.5%	79.4%
Terminal rates	0/24 (0%)	0/15 (0%)	4/26 (15%)	0/1 (0%)
First incidence (days)	641	651	403	512
Logistic regression tests	P<0.001	P=0.376	P=0.112	P<0.001
Liver: Cystic Degeneration				
Overall rates	6/50 (12%)	13/35 (37%)	33/65 (51%)	31/50 (62%)
Adjusted rates	19.7%	60.0%	85.7%	100.0%
Terminal rates	3/24 (13%)	7/15 (47%)	21/26 (81%)	1/1 (100%)
First incidence (days)	454	530	565	470
Logistic regression tests	P<0.001	P=0.006	P<0.001	P<0.001
Liver: Hepatocyte Necrosis				
Overall rates	3/50 (6%)	3/35 (9%)	10/65 (15%)	22/50 (44%)
Adjusted rates	7.7%	12.6%	26.1%	88.6%
Terminal rates	0/24 (0%)	0/15 (0%)	3/26 (12%)	0/1 (0%)
First incidence (days)	454	447	654	412
Logistic regression tests	P<0.001	P=0.471	P=0.099	P<0.001
Liver: Hepatocyte Fatty Change				
Overall rates	3/50 (6%)	3/35 (9%)	6/65 (9%)	7/50 (14%)
Adjusted rates	8.4%	11.8%	14.9%	43.5%
Terminal rates	1/24 (4%)	0/15 (0%)	1/26 (4%)	0/1 (0%)
First incidence (days)	355	447	567	473
Logistic regression tests	P=0.197	P=0.456	P=0.356	P=0.226

TABLE 15
Nonneoplastic Liver Lesions in F344/N Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114
(continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Female				
Liver: Eosinophilic Focus				
Overall rates	0/50 (0%)	12/35 (34%)	38/64 (59%)	42/50 (84%)
Adjusted rates	0.0%	63.1%	95.4%	100.0%
Terminal rates	0/36 (0%)	7/13 (54%)	5/6 (83%)	0/0 (0%)
First incidence (days)	–	538	400	258
Logistic regression tests	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Mixed Cell Focus				
Overall rates	2/50 (4%)	9/35 (26%)	8/64 (13%)	15/50 (30%)
Adjusted rates	5.6%	51.8%	53.3%	76.8%
Terminal rates	2/36 (6%)	6/13 (46%)	2/6 (33%)	0/0 (0%)
First incidence (days)	733 (T)	523	538	186
Logistic regression tests	P=0.001	P=0.002	P=0.014	P=0.053
Liver: Hematopoietic Cell Proliferation				
Overall rates	0/50 (0%)	7/35 (20%)	15/64 (23%)	9/50 (18%)
Adjusted rates	0.0%	30.2%	59.1%	49.9%
Terminal rates	0/36 (0%)	2/13 (15%)	2/6 (33%)	0/0 (0%)
First incidence (days)	–	411	400	339
Logistic regression tests	P=0.058	P=0.006	P<0.001	P=0.041
Liver: Cystic Degeneration				
Overall rates	0/50 (0%)	5/35 (14%)	25/64 (39%)	14/50 (28%)
Adjusted rates	0.0%	25.2%	88.3%	71.5%
Terminal rates	0/36 (0%)	1/13 (8%)	4/6 (67%)	0/0 (0%)
First incidence (days)	–	638	521	339
Logistic regression tests	P<0.001	P=0.010	P<0.001	P<0.001
Liver: Hepatocyte Necrosis				
Overall rates	2/50 (4%)	3/35 (9%)	9/64 (14%)	8/50 (16%)
Adjusted rates	4.0%	18.4%	40.3%	53.7%
Terminal rates	0/36 (0%)	1/13 (8%)	1/6 (17%)	0/0 (0%)
First incidence (days)	431	663	496	355
Logistic regression tests	P=0.069	P=0.488	P=0.139	P=0.566
Liver: Hepatocyte Fatty Change				
Overall rates	2/50 (4%)	4/35 (11%)	10/64 (16%)	8/50 (16%)
Adjusted rates	5.6%	15.6%	37.2%	100.0%
Terminal rates	2/36 (6%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	733 (T)	462	477	351
Logistic regression tests	P=0.119	P=0.247	P=0.077	P=0.055

(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals necropsied or examined microscopically for this tumor type
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the "0 ppm" column are the P values associated with the trend test. Beneath the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal.

TABLE 16
Proliferative Lesions of the Lung in F344/N Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114

Male	0 ppm	70 ppm	150 ppm	300 ppm
Lung: Alveolar Epithelial Hyperplasia				
Overall rates[a]	2/50 (4%)	4/35 (11%)	9/65 (14%)	8/50 (16%)
Lung: Alveolar/bronchiolar Adenoma				
Overall rates	0/50 (0%)	2/35 (6%)	1/65 (2%)	3/50 (6%)
Effective rates[b]	0/35 (0%)	2/23 (9%)	1/48 (2%)	3/18 (17%)
Terminal rates[c]	0/24 (0%)	1/15 (7%)	0/26 (0%)	1/1 (100%)
First incidence (days)	–[d]	701	654	694
Logistic regression tests[e]	P=0.022	P=0.148	P=0.550	P=0.002
Lung: Alveolar/bronchiolar Carcinoma				
Overall rates	2/50 (4%)	0/35 (0%)	1/65 (2%)	0/50 (0%)
Lung: Alveolar/bronchiolar Adenoma or Carcinoma[f]				
Overall rates	2/50 (4%)	2/35 (6%)	2/65 (3%)	3/50 (6%)
Effective rates	2/35 (6%)	2/23 (9%)	2/48 (4%)	3/18 (17%)
Terminal rates	2/24 (8%)	1/15 (7%)	1/26 (4%)	1/1 (100%)
First incidence (days)	732 (T)	701	654	694
Logistic regression tests	P=0.102	P=0.521	P=0.618N	P=0.017

Female	0 ppm	150 ppm	300 ppm	600 ppm
Lung: Alveolar Epithelium, Hyperplasia				
Overall rates	6/50 (12%)	6/35 (17%)	15/65 (23%)	20/50 (40%)**
Lung: Alveolar/bronchiolar Adenoma				
Overall rates	1/50 (2%)	2/35 (6%)	8/65 (12%)	4/50 (8%)
Effective rates	1/50 (2%)	2/35 (6%)	8/59 (14%)	4/31 (13%)
Terminal rates	1/36 (3%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	733 (T)	411	582	454
Logistic regression tests	P=0.013	P=0.508	P=0.003	P=0.120
Lung: Alveolar/bronchiolar Carcinoma				
Overall rates	0/50 (0%)	0/35 (90%)	1/65 (2%)	0/50 (0%)
Lung: Alveolar/bronchiolar Adenoma or Carcinoma[g]				
Overall rates	1/50 (2%)	2/35 (6%)	9/65 (14%)	4/50 (8%)
Effective rates	1/50 (2%)	2/35 (6%)	9/59 (15%)	4/31 (13%)
Terminal rates	1/36 (3%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	733 (T)	411	582	454
Logistic regression tests	P=0.007	P=0.508	P=0.001	P=0.120

(T)Terminal sacrifice
** Significantly different (P≤0.01) from the control group by the logistic regression test.
[a] Number of lesion-bearing animals/number of animals necropsied or examined microscopically for this tumor type
[b] Number of lesion-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this tumor type in any of the groups
[c] Observed incidence at terminal kill
[d] Not applicable; no tumors in animal group
[e] Beneath the "0 ppm" column are the P values associated with the trend test. Beneath the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal. For all tests, a negative trend or a lower incidence in a dosed group is indicated by N.
[f] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 22/680 (3.2% ± 2.6%); range 0%-10%
[g] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 13/679 (1.9% ± 2.1%); range 0%-6%

Oral Cavity (Tongue or Pharynx): Squamous cell papillomas and carcinomas, uncommon neoplasms in control F344/N rats, were moderately increased in dosed females compared to controls; three squamous cell papillomas occurred in dosed males (Table 17). However, the incidence was significantly increased only in the mid-dose female group. Squamous cell papillomas were exophytic masses arising from the oral mucosa and consisted of a pedunculated, highly branched core of fibrous tissue covered by a thick layer of stratified squamous epithelium. Squamous cell carcinomas were flat, broad lesions of the oral mucosa that consisted of cords and clusters of disorganized, pleomorphic, squamous epithelial cells that invaded deep into the underlying submucosa. Fibroplasia and inflammation sometimes accompanied the invasion.

Small Intestine (Duodenum or Jejunum): A few neoplasms of the small intestine occurred in female rats treated with C.I. Acid Red 114. An adenomatous polyp was found in one mid-dose and in one high-dose female, while an adenocarcinoma occurred in one high-dose female. These neoplasms are rare in F344/N rats. No adenomatous polyps or carcinomas were found in 680 untreated female controls from NTP 2-year studies (Table B4l). A single adenocarcinoma occurred in a high-dose male. Although the incidence of neoplasms in female rats is low, the fact that these neoplasms are rare plus the fact that two occurred in the high-dose group in which there was markedly reduced survival suggest that these neoplasms may have been chemical related. The adenomatous polyps were pedunculated, exophytic masses consisting of a stalk-like core of fibrous tissue and covered by numerous glandular structures and were lined by a single layer of well-differentiated, columnar cells with abundant basophilic cytoplasm. Adenocarcinomas were poorly demarcated and invaded the submucosal and muscular layers of the intestinal wall. They consisted of large, poorly differentiated, columnar cells that formed multiple, irregular, variably sized glandular structures surrounded by abundant fibrous tissue stroma, and contained large cystic spaces filled with mucus and debris (cystic mucinous adenocarcinoma).

Large Intestine (Colon or Rectum): Adenomatous polyps were seen in two high-dose and one low-dose female, and an adenocarcinoma was noted in a single high-dose female. These neoplasms are rare in F344/N rats. No adenomatous polyps or adenocarcinomas of the large intestine were found in 680 untreated female control rats from NTP 2-year studies (Table B4l). Although the number of large intestine neoplasms is small, the fact that these are rare neoplasms plus the fact that three occurred in the high-dose group in which there was markedly reduced survival suggest these neoplasms may have been related to chemical administration. Neoplasms in the large intestine had a similar histologic appearance to those seen in the small intestine.

Mammary Gland: Adenocarcinomas of the mammary gland occurred in treated groups of female rats (Table 18). The incidences fall within the historical control range for untreated female F344/N rats from NTP 2-year studies (Table B4i). The incidence in the mid-dose group compared to controls was significant by the Fisher exact test using effective rates. Mammary gland adenocarcinomas may be fatal neoplasms; however, eight of the 12 dosed females with mammary gland adenocarcinomas also had one or more neoplasms that could have been the cause of death. Thus, for this neoplasm the logistic regression or Fisher exact test were considered more appropriate. Although all incidences fall within the historical control range, the fact that these neoplasms occurred only in dosed females, and that the incidence was significantly increased in the mid-dose group, indicate these neoplasms may have been treatment related. Histologically, adenocarcinomas invaded adjacent tissue and consisted of acinar structures and solid nodules of cuboidal to columnar cells with vacuolated cytoplasm and pleomorphic, deeply basophilic nuclei. The incidence of mammary gland adenoma was increased in the mid-dose group as compared with untreated controls. The increase was not statistically significant, but the incidence in the mid-dose group was slightly above the historical control range for NTP 2-year drinking water studies (Table B4i). Fibroadenomas occurred at decreased incidences in mid- and high-dose females. This may have been a reflection of the decreased survival in these groups.

Adrenal Gland: In the adrenal medulla, the combined incidence of benign or malignant pheochromocytomas was significantly increased in the high-dose males (17/50, 34%; 11/35, 31%; 27/63, 43%; 21/49, 43%). However, these incidences are within the historical control incidence for untreated male F344/N rats from NTP 2-year studies (Table A4i). The incidences of focal or multifocal hyperplasia, the precursor lesion to pheochromocytoma, were significantly ($P<0.05$) increased in mid-dose males (12/50, 24%; 7/35, 20%; 28/63, 44%; 13/49, 27%). Pheochromocytomas occurred in small numbers of females in all groups, but were more frequent in the low- and mid-dose groups. The

TABLE 17
Oral Cavity Neoplasms in F344/N Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114

Male	0 ppm	70 ppm	150 ppm	300 ppm
Oral Cavity: Squamous Cell Papilloma				
Overall rates[a]	0/50 (0%)	0/35 (0%)	1/65 (2%)	2/50 (4%)
Oral Cavity: Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	0/65 (0%)	0/50 (0%)
Oral Cavity: Squamous Cell Papilloma or Squamous Cell Carcinoma[b]				
Overall rates	0/50 (0%)	0/35 (0%)	1/65 (2%)	2/50 (4%)

Female	0 ppm	150 ppm	300 ppm	600 ppm
Oral Cavity: Squamous Cell Papilloma				
Overall rates	0/50 (0%)	3/35 (9%)	6/65 (9%)	4/50 (8%)
Effective rates[c]	0/50 (0%)	3/35 (9%)	6/61 (10%)	4/44 (9%)
Terminal rates[d]	0/36 (0%)	2/13 (15%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–[e]	567	487	349
Logistic regression tests[f]	P=0.193	P=0.059	P=0.066	P=0.302
Cochran-Armitage test[f]	P=0.076			
Fisher exact test[f]		P=0.066	P=0.025	P=0.045
Oral Cavity: Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	3/65 (5%)	2/50 (4%)
Effective rates	0/50 (0%)	0/35 (0%)	3/61 (5%)	2/40 (5%)
Terminal rates	0/36 (0%)	0/13 (0%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	–	454	372
Logistic regression tests	P=0.408	–	P=0.295	P=0.795
Cochran-Armitage test	P=0.077			
Fisher exact test		–	P=0.162	P=0.195
Oral Cavity: Squamous Cell Papilloma or Squamous Cell Carcinoma[g]				
Overall rates	0/50 (0%)	3/35 (9%)	9/65 (14%)	6/50 (12%)
Effective rates	0/50 (0%)	3/35 (9%)	9/61 (15%)	6/44 (14%)
Terminal rates	0/36 (0%)	2/13 (15%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	567	454	349
Logistic regression tests	P=0.156	P=0.059	P=0.025	P=0.225
Cochran-Armitage test	P=0.017			
Fisher exact test		P=0.066	P=0.003	P=0.009

[a] Number of lesion-bearing animals/number of animals necropsied or examined microscopically for this tumor type
[b] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 4/681 (0.6% ± 1.5%); range 0%-4%
[c] Number of lesion-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this tumor type in any of the groups
[d] Observed incidence at terminal kill
[e] Not applicable; no tumors in animal group
[f] Beneath the "0 ppm" column are the P values associated with the trend test. Beneath the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the effective incidence rates.
[g] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 6/680 (0.9% ± 1.0%); range 0%-2%

TABLE 18
Mammary Gland Neoplasms in Female F344/N Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

	0 ppm	150 ppm	300 ppm	600 ppm
Mammary Gland: Adenocarcinoma[a]				
Overall rates[b]	0/50 (0%)	3/35 (9%)	6/65 (9%)	3/50 (6%)
Effective rates[c]	0/50 (0%)	3/35 (9%)	6/63 (10%)	3/46 (7%)
Terminal rates[d]	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–[e]	582	285	351
Logistic regression tests[f]	P=0.456	P=0.089	P=0.061	P=0.755
Cochran-Armitage test[f]	P=0.170			
Fisher exact test[f]		P=0.066	P=0.027	P=0.106
Mammary Gland: Adenoma[g]				
Overall rates	1/50 (2%)	1/35 (3%)	4/65 (6%)	0/50 (0%)
Effective rates	1/46 (2%)	1/30 (3%)	4/45 (9%)	0/8 (0%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	711	663	538	–
Logistic regression tests	P=0.433	P=0.672	P=0.166	P=0.999N
Cochran-Armitage test[f]	P=0.363			
Fisher exact test		P=0.637	P=0.174	P=0.852N
Mammary Gland: Fibroadenoma[h]				
Overall rates	19/50 (38%)	13/35 (37%)	12/65 (18%)	1/50 (2%)
Effective rates	19/46 (41%)	13/30 (43%)	12/45 (27%)	1/8 (13%)
Terminal rates	15/36 (42%)	9/13 (69%)	4/6 (67%)	0/0 (0%)
First incidence (days)	683	638	538	603
Logistic regression tests	P=0.394	P=0.194	P=0.466	P=0.641
Cochran-Armitage test[f]	P=0.033N			
Fisher exact test		P=0.524	P=0.105N	P=0.121N

[a] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 22/680 (3.2% ± 4.0%); range 0%-12%
[b] Number of lesion-bearing animals/number of animals necropsied or examined microscopically for this tumor type
[c] Number of lesion-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this tumor type in any of the groups
[d] Observed incidence at terminal kill
[e] Not applicable; no tumors in animal group
[f] Beneath the "0 ppm" column are the P values associated with the trend test. Beneath the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage test and Fisher exact tests compare directly the effective incidence rates. For all tests, a negative trend or a lower incidence in a dosed group is indicated by N.
[g] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 7/680 (1.0% ± 1.3%); range 0%-4%
[h] Historical incidence for 2-year studies for untreated control groups in NTP feed and drinking water studies (mean ± standard deviation): 235/680 (34.6% ± 13.2%); range 8%-56%

incidence of hyperplasia was significantly ($P<0.05$) increased in mid-dose females as compared with control: for pheochromocytomas, 1/50 (2%), 3/35 (9%), 5/64 (8%), 1/50 (2%); for hyperplasia, 6/50 (12%), 7/35 (20%), 15/64 (22%), 2/50 (4%). The incidence of pheochromocytomas in all dose groups is within the historical control incidence for untreated female F344/N rats from NTP 2-year studies (Table B4k). The low incidence of hyperplasia in high-dose females is presumably a reflection of the decreased survival in this group. The significance of these findings is unclear, but evidence of a significant increase in the high-dose male group despite the decreased survival, and the increases in hyperplasia and neoplastic lesions in low- and mid-dose females, suggest a possible treatment-related effect.

Hematopoietic System: The incidences of mononuclear cell leukemia in dosed female rats were increased relative to controls (12/50, 24%; 13/35, 37%; 18/65, 28%; 5/50, 10%). Mononuclear cell leukemia occurs commonly in control female rats, and the incidence in the low-dose group just exceeds the historical control range for untreated control females from NTP 2-year studies (Table B4n). A similar increase was not observed in the mid- or high-dose groups. The incidences in all dosed groups were significantly different from controls by the life table test. Since mononuclear cell leukemia is considered rapidly lethal, the life table test is usually the most appropriate statistical analysis for these neoplasms. However, in this study there were several other types of malignant neoplasms which could have caused the deaths of the animals. Thus, due to the competing risks from other lethal neoplasms, the life table test may not be the most appropriate analysis in this case. Accordingly, it is unclear whether or not the slight increase in mononuclear cell leukemia in treated females was related to chemical administration.

Thyroid Gland: Follicular cell adenoma or carcinoma (combined) occurred with low incidences in dosed female rats (control, 0/50; low-dose, 3/35, 9%; mid-dose, 3/64, 5%; high-dose, 1/50, 2%). The incidence in the low-dose group was significantly different from controls by logistic regression analysis ($P=0.011$) and fell outside the range of historical controls for follicular cell neoplasms in untreated female rats from NTP 2-year studies (Table B4j). The incidences of follicular cell hyperplasia, a lesion generally considered to be a precursor to follicular cell neoplasia, were similar among female groups (1/50, 2%; 1/35, 3%; 3/64, 5%; 2/50, 4%). Incidences of follicular cell neoplasms or hyperplasia were not increased in dosed male rats. The biological significance of the follicular cell neoplasms in dosed female rats is questionable.

Kidney: Nephropathy, a spontaneous age-related disease, occurred in nearly all female rats. However, the nephropathy was more severe in high-dose females than in controls. Severity was graded by the fraction of renal parenchyma affected, as follows: minimal, usually less than 20%; mild, 20% to 50%; moderate, 50% to 75%; marked, greater than 75%. When expressed on a scale of one to four (1=minimal, 2=mild, 3=moderate, 4=marked); the average severity per group was control, 1.9; low-dose, 1.9; mid-dose, 2.1; and high-dose, 2.8. Nephropathy consisted of a spectrum of lesions including varying degrees of tubule dilatation and distortion with occasional cyst formation; proteinaceous tubule casts; atrophy, regeneration, and hypertrophy of tubule epithelium; thickening of tubule and glomerular basement membranes; interstitial fibrosis; scattered foci of suppurative inflammation, primarily within degenerate tubules; and a scattering of varying numbers of mononuclear inflammatory cells within the interstitium. Karyomegaly (enlargement of tubule epithelial cells), was diagnosed in 11/50 high-dose females; this change may have been secondary to the nephropathy. Parathyroid gland hyperplasia and fibrous osteodystrophy of the bone occurred in some high-dose females secondary to alteration in calcium metabolism caused by the nephropathy.

Uterus: The incidence of endometrial stromal polyps was significantly increased in the low-dose group compared with the controls (4/50, 8%; 8/35, 23%; 8/65, 12%; 2/50, 4%). However, endometrial stromal polyps are common in female F344/N rats and the incidence in the low-dose group is within the historical control range for untreated F344/N females from NTP 2-year studies (Table B4m). Also, the incidence in the control group from this study was at the low end of the historical control range. Consequently, the increased incidence of endometrial stromal polyps in the low-dose group is not considered to be a treatment-related effect.

Spleen: Hematopoietic cell proliferation occurred with increasing incidences in the spleens of treated male and female rats (males: 3/50, 4/35, 3/64, 21/50; females: 3/50, 8/35, 20/63, 16/50). This effect was considered to be secondary to the mild anemia and to the inflammation associated with neoplasms in treated animals.

Heart: The incidence of thrombus within the atrium of the heart was increased in the mid- and high-dose male groups as compared with controls (5/50, 4/35, 18/65, 18/50). Although the biological significance of the thrombi is unclear, it is possible they may have formed as a consequence of debilitation in tumor-bearing animals. Debilitation may have led to impaired circulation which allowed pooling of blood within the atrium, resulting in thrombus formation.

GENETIC TOXICOLOGY

C.I. Acid Red 114 was tested for induction of gene mutations in *Salmonella typhimurium* by a standard preincubation protocol at concentrations of 100 to 10,000 μg/plate in the presence and the absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table C1; Mortelmans *et al.*, 1986). A weakly positive response was observed in TA98 with hamster S9 and an equivocal response was observed in TA100, also in the presence of hamster S9. No significant mutagenic activity was observed in TA1535 or TA1537. This compound, as with most benzidine congener dyes, requires reductive metabolism of the azo bonds to release the parent amine, which then can be oxidatively metabolized to an active mutagen. When tested by such a reductive/oxidative metabolism protocol, C.I. Acid Red 114 was highly mutagenic to *S. typhimurium* strain TA1538 (Table C2; Reid *et al.*, 1984a). Some mutagenic activity was observed in the presence of rat S9 without prior reduction, but the mutagenicity was greatly enhanced following reduction. With both reduction systems (cecal bacteria and flavin mononucleotide) the mutagenic response obtained with C.I. Acid Red 114 was much greater than expected based on the activity of equimolar amounts of the parent diamine (dimethylbenzidine). This may indicate the presence of mutagenic impurities and the formation of additional reduction products in the crude dye mixture that was tested.

In cytogenetic tests with Chinese hamster ovary cells, C.I. Acid Red 114 did not induce sister chromatid exchanges (Table C3) or chromosomal aberrations (Table C4) when tested at concentrations up to 160 μg/mL, with or without Aroclor 1254-induced male Sprague-Dawley rat liver S9. Reductive metabolism was not used in these cytogenetic tests.

No increase in sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* following administration of C.I. Acid Red 114 either in feed (50,000 ppm) or by injection (1,500 ppm) (Table C5; Zimmering *et al.*, 1985).

DISCUSSION AND CONCLUSIONS

The benzidine dyes and their parent congeners are widely used in manufacturing throughout the United States. The potential health hazard in the workplace from excessive exposure to the benzidine congener dyes was considered significant enough that the Benzidine Dye Initiative was established through NTP in cooperation with NIEHS, NCTR, NIOSH, USEPA, OSHA, and the Consumer Product Safety Commission (NIOSH, 1981; NIOSH, 1983). Since there are more than 90 benzidine-based dyes in use, the Initiative focused on studying the metabolism, pharmacokinetics, genetic toxicology, and *in vivo* carcinogenicity of several representative dyes. The five selected for developing a toxicity and carcinogenic activity data base were C.I. Acid Red 114 and its parent congener 3,3′-dimethylbenzidine, and C.I. Direct Blue 15, C.I. Direct Blue 218, and their parent congener, 3,3′-dimethoxybenzidine. The route of chemical administration selected for these studies in male and female rats was through the drinking water to ensure systemic exposure. The toxicology and carcinogenicity studies of 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine, and C. I. Direct Blue 15 have been reported (NTP, 1990, 1991, in press). The studies described in this report examine the toxic and carcinogenic properties of C.I. Acid Red 114 (desalted industrial grade), the fourth chemical studied under the Benzidine Dye Initiative.

No treatment-related deaths occurred in either the 13-day or 13-week studies. Body weight reduction, water consumption, organ weight changes, hematologic changes, and significant histopathologic findings in the 13-day and 13-week studies were considered in selecting the dose levels of C.I. Acid Red 114 for the 2-year studies. Body weights were significantly decreased relative to the controls in males at the 20,000 and 30,000 ppm dose levels and in all female dose groups in the 13-day studies. In rats receiving doses of 1,200 ppm or higher during the 13-week studies, decreases in body weights relative to the controls ranged from 11% to 15% for males and from 6% to 11% for females. Water consumption was markedly lower in dosed animals in the 13-day and 13-week studies than in the controls. In the 13-week studies, both absolute and relative liver weights were significantly increased for all male and female dose groups compared to the controls.

Bone marrow of male and female rats receiving 20,000 ppm or higher C.I. Acid Red 114 for 13 days was depleted of erythroid and myeloid cells, suggesting there was a direct cytotoxic effect that resulted in anemia at these dose levels. The mechanism of the cytotoxic effect is unknown but may be due, in part, to the binding of benzidine metabolites to DNA (Zenser *et al.*, 1980; Yamazoe *et al.*, 1988).

In the 13-week studies, treatment-related decreases in hematocrit, hemoglobin, and erythrocyte counts were observed in females and to a lesser extent in males. The parent congener of C.I. Acid Red 114, 3,3′-dimethylbenzidine, showed similar results in high-dose groups after 13 weeks of chemical administration (NTP, 1991). 3,3′-dimethoxybenzidine and C.I. Direct Blue 15 caused more modest changes in the hematology profile than did 3,3′-dimethylbenzidine (NTP, 1990, in press).

Serum levels of alanine aminotransferase, lactate dehydrogenase, and sorbitol dehydrogenase in dosed males were elevated, characteristic of liver damage. In females, sorbitol dehydrogenase was the primary enzyme with elevated serum levels which suggests the liver damage was less severe. This conclusion was confirmed to a limited extent by histopathologic findings in which male livers had a centrilobular pallor while female livers were only pigmented. Elevated levels of sorbitol dehydrogenase also occurred in animals administered 3,3′-dimethylbenzidine for 13 weeks but only a marginal elevation of liver enzyme levels was present in animals administered 3,3′-dimethoxybenzidine or C.I. Direct Blue 15 after 13 weeks (NTP 1990, 1991, in press).

In female rats receiving 2,500 ppm or higher for 13 weeks, kidneys developed degenerative lesions consisting of inflammation, tubule regeneration, and karyomegaly. Similar lesions were observed in male and female rats after 13 weeks of 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine, and C.I. Direct

Blue 15 administration (NTP 1990, 1991, in press). Pancreatic acinar cell degeneration was noted in dosed male and female rats as it had been noted in previous benzidine dye studies; however, its occurrence may be secondary as a result of the general debilitation of the study animals.

Based on the results of the 13-day and 13-week studies, the dose levels of C.I. Acid Red 114 selected for males in the 2-year studies were 0, 70, 150, and 300 ppm, and dose levels selected for females were 0, 150, 300, and 600 ppm. After 2 years, treatment-related tumors were found in many sites of male and female rats including skin, Zymbal's gland, liver, oral cavity, lung, and adrenal gland. Tumors occurring only in female rats were found in the clitoral gland, small and large intestines, and mammary gland. At 9- or 15-month interim evaluations, tumors were found in the liver, lung, clitoral gland, skin, Zymbal's gland, oral cavity, and intestine. The unusually early appearance of these tumors demonstrates the carcinogenic potency of C.I. Acid Red 114.

The incidence of skin basal cell neoplasms increased in male rats (control, 1/50; 70 ppm, 5/35; 150 ppm, 28/65; 300 ppm, 32/50) and to a lesser extent in female rats (0/50; 4/35; 7/65; 5/50). The higher incidence of skin basal cell tumors in male rats was also seen in the 3,3′-dimethylbenzidine, 3,3′dimethoxybenzidine, and C.I. Direct Blue 15 studies (NTP 1990, 1991, in press). The mechanism for this sex difference could not be determined but may be due, in part, to metabolic differences between the sexes.

As in the previous three Benzidine Dye Initiative studies, the incidences of Zymbal's gland neoplasms in male and female rats were increased by C.I. Acid Red 114 exposure. In the NTP database of over 350 rodent studies, 17 chemicals induced Zymbal's gland tumors, 14 induced skin tumors, and 11 induced both tumors in rats (Table 19). Most of the chemicals inducing either Zymbal's gland or skin neoplasms also caused neoplasms at other sites. These tumor-inducing chemicals have a common aromatic-amine grouping which is considered to be a "structural alert" for genotoxic activity (Ashby and Tennant, 1988). This is supported by positive mutagenicity results in *Salmonella typhimurium* assays in the C.I. Acid Red 114 studies as well as the 3,3′-dimethylbenzidine, 3,3′dimethoxybenzidine, and C.I. Direct Blue 15 studies.

In female rats, the incidence of clitoral gland adenomas and carcinomas was increased as in the previous three benzidine-congener dye studies. A similar dose-related increase in preputial gland neoplasms was not found in male rats receiving C.I. Acid Red 114, but was found in male rats receiving 3,3′-dimethylbenzidine, 3,3′dimethoxybenzidine, and C.I. Direct Blue 15. The reason male rats were not susceptible to preputial gland neoplasms following 2 years of C.I. Acid Red 114 administration is not known.

The incidence of liver neoplasms was increased in dosed male rats and, to a lesser extent, in dosed female rats. The higher incidences of liver neoplasms in dosed male rats relative to females was also observed in the 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine, and C.I. Direct Blue 15 studies. Whether the differences in the incidences of liver neoplasms are related to differences between the sexes in metabolizing benzidine compounds, or are the result of some other mechanism, has not been determined.

There was a marginal but statistically significant increase in the combined incidence of alveolar/bronchiolar adenomas or carcinomas in the treated male rats. While the numbers for these neoplasms are low, dimethylbenzidine, the parent congener, also caused an increase in neoplasms at this site in male rats.

The incidences of neoplasms in the oral cavity and in the small or large intestine of female rats were also above the incidences in the concurrent control and the historical database. In male rats receiving C.I. Acid Red 114, the incidence of oral cavity neoplasms was marginally increased compared to the controls; no statistically significant increases in the incidence of intestine tumors occurred. In the previous benzidine congener dye studies, both male and female rats had increased incidences of oral cavity and intestinal neoplasms.

The combined incidences of benign or malignant pheochromocytomas of the adrenal medulla were marginally increased in male and female rats. The incidences were higher in the low- or mid-dose groups, and this was attributed to the early death of most of the animals at the high dose. Supportive evidence for a neoplastic response in this organ was the increased incidence of focal or multifocal hyperplasia.

TABLE 19
Evidence of Zymbal's Gland and Skin Tumors in Rats and *Salmonella* Mutagenicity for Selected National Toxicology Program Chemicals[a]

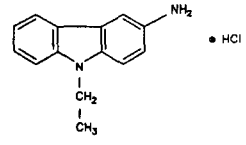
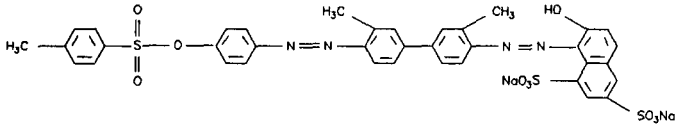
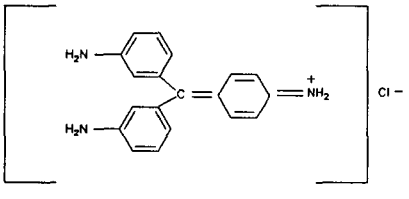
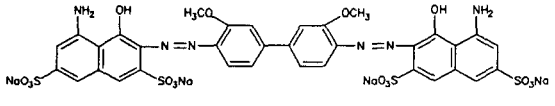
Chemical Name/ Structure	Technical Report Number	Zymbal's Gland Tumors M F	Skin Tumors M F	*Salmonella* Mutagenicity Results
3-Amino-9-Ethylcarbazole HCl 	93	+ +	+	+
Benzene 	289	+ +	+	–
C.I. Acid Red 114 	405	+ +	+ +	+
C.I. Basic Red 9 Monohydrochloride 	285	+ +	+	+
C.I. Direct Blue 15 	397	+ +	+ +	+

TABLE 19
Evidence of Zymbal's Gland and Skin Tumors in Rats and *Salmonella* Mutagenicity for Selected National Toxicology Program Chemicals (continued)

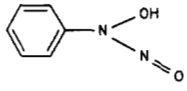
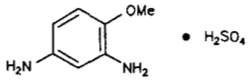
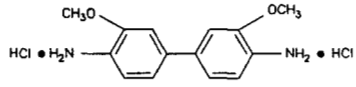
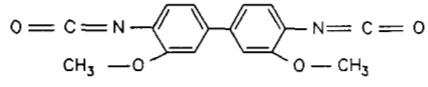
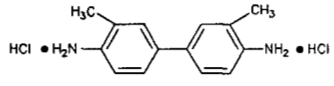
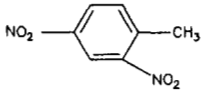
Chemical Name/ Structure	Technical Report Number	Zymbal's Gland Tumors M F	Skin Tumors M F	*Salmonella* Mutagenicity Results
Cupferron 	100	+		+
2,4-Diaminoanisole Sulfate 	84	+ +	+	+
3,3′-Dimethoxybenzidine Dihydrochloride 	372	+ +	+ +	+
3,3′-Dimethoxybenzidine-4,4′-Diisocyanate 	128	+ +	+	+
3,3′-Dimethylbenzidine Dihydrochloride 	390	+ +	+ +	+
2,4-Dinitrotoluene 	54		+	+

TABLE 19
Evidence of Zymbal's Gland and Skin Tumors in Rats and *Salmonella* Mutagenicity for Selected National Toxicology Program Chemicals (continued)

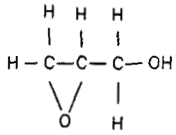
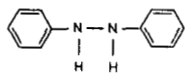
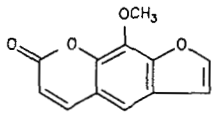
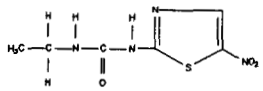
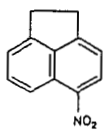
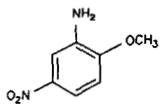
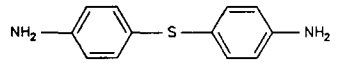
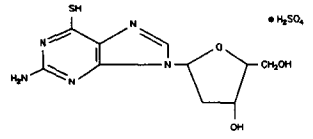
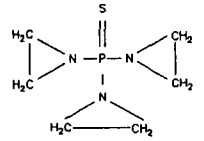
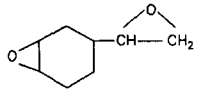
Chemical Name/ Structure	Technical Report Number	Zymbal's Gland Tumors M F	Skin Tumors M F	*Salmonella* Mutagenicity Results
Glycidol 	374	+	+	+
Hydrazobenzene 	92	+		+
8-Methoxypsoralen 	359	+		+
Nithiazide 	146		+	+
5-Nitroacenaphthene 	118	+ +		+
5-Nitro-*o*-Anisidine 	127	+ +	+	+

TABLE 19
Evidence of Zymbal's Gland and Skin Tumors in Rats and *Salmonella* Mutagenicity for Selected National Toxicology Program Chemicals (continued)

Chemical Name/ Structure	Technical Report Number	Zymbal's Gland Tumors M F	Skin Tumors M F	*Salmonella* Mutagenicity Results
4,4′-Thiodianiline	47	+ +		+
				
β-Thioguanidine Deoxyriboside	57	+		
				
Tris(Aziridinyl)Phosphine Sulfide	58	+ +	+ +	+
				
4-Vinyl-1-Cyclohexene Diepoxide	362		+ +	+
				

[a] + = positive evidence or results; − = negative results

The incidence of adenocarcinoma of the mammary gland in female rats was significantly increased in the mid-dose group. Mammary gland neoplasms were also seen in female rats after treatment with 3,3′-dimethoxybenzidine, 3,3′-dimethylbenzidine, and C.I. Direct Blue 15. Mononuclear cell leukemia was also considered to be marginally increased in female rats.

The spectrum of lesions found in the skin, Zymbal's gland, liver, oral cavity, clitoral gland, and to a lesser extent the small and large intestine was similar for C.I. Acid Red 114, 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine, and C.I. Direct Blue 15. The incidences of skin and liver tumors were higher in male than in female rats in all four benzidine-congener dye studies. However, the incidences of liver tumors produced by administration of C.I. Acid Red 114 and its parent congener 3,3′-dimethylbenzidine was greater than the tumor incidences produced by C.I. Direct Blue 15 or its parent congener, 3,3′dimethoxybenzidine. Some, but not all of the four, benzidine chemicals studied caused increases in the occurrence of lesions in the brain, mammary gland, lung, and adrenal gland as well as mononuclear cell leukemia and mesotheliomas. Clear evidence for increased incidence of preputial gland tumors was seen in the studies of

3,3′-dimethylbenzidine, C.I. Direct Blue 15, and 3,3′-dimethoxybenzidine, but not in the studies of C.I. Acid Red 114.

Earlier studies showed carcinogenic activity in rats from exposure to the benzidine congeners, 3,3′-dimethylbenzidine and 3,3′-dimethoxybenzidine. Hadidian *et al.* (1968) found evidence for treatment-related neoplasms in the urinary bladder, mammary gland, intestinal tract, and Zymbal's gland of male and female rats after 52 weeks of 3,3′-dimethoxybenzidine administered by gavage at doses ranging from 0.3 to 10 mg/kg. The number of animals treated was small (3 to 15/dose group), so it was necessary to pool the data to determine any treatment-related effects. In hamsters, 3,3′-dimethoxybenzidine induced urinary bladder tumors and forestomach papillomas (Saffiotti *et al.*, 1967; Sellakumar *et al.*, 1969). In previous studies on the carcinogenic effects of 3,3′-dimethylbenzidine, rats developed Zymbal's gland, preputial gland, and mammary gland tumors after receiving subcutaneous injections for 2 to 14 months (Spitz *et al.*, 1950; Pliss and Zabezhinsky, 1970).

The carcinogenic effects of the benzidine compounds have been studied in mice as well. The National Center for Toxicological Research conducted studies on the toxic and carcinogenic activities of 3,3′-dimethylbenzidine dihydrochloride and 3,3′dimethoxybenzidine dihydrochloride in the BALB/c mouse (Schieferstein *et al.*, 1989, 1990). The BALB/c mouse was selected because it is susceptible to chemically induced cancer of the urinary bladder, which is the target organ for benzidine carcinogenicity in humans (Meigs *et al.*, 1986). 3,3′-Dimethoxybenzidine at doses from 20 to 630 ppm or 3,3′-dimethylbenzidine at doses from 5 to 140 ppm was administered to BALB/c mice in drinking water for 112 to 116 weeks. Results showed decreased water consumption and lower body weights in male and female mice given 3,3′-dimethoxybenzidine and in female mice given 3,3′-dimethylbenzidine. Unlike the rat studies, BALB/c mice produced no treatment-related neoplasms in the Zymbal's gland, skin, oral cavity, preputial gland, clitoral gland, intestine, or liver, although increased incidences in lung neoplasms were seen in male mice treated with 3,3′-dimethylbenzidine (Schieferstein *et al.*, 1989, 1990). Vesselinovitch *et al.* (1975) reported that benzidine caused liver tumors, lung adenomas, and harderian gland tumors in B6C3F$_1$ mice. Other studies of benzidine showed treatment-related liver tumors in mice (Littlefield *et al.*, 1983) and in rats (Spitz *et al.*, 1950; Pliss and Zabezhinsky, 1970).

Weisburger (1983) reported that rats were more sensitive than mice to the carcinogenic effects of aromatic amines. Differences in absorption and metabolism and in experimental design might account for the different susceptibility of rats and mice to the carcinogenic effects from benzidine, the benzidine congeners, and benzidine dyes. There is limited information on the absorption of 3,3′-dimethoxybenzidine, 3,3′-dimethylbenzidine, C.I. Direct Blue 15, and C.I. Acid Red 114 in rats after oral administration. Lynn *et al.* (1980) showed that only small amounts of C.I. Direct Blue 15 or C.I. Acid Red 114 (less than 1% of the administered dose) were excreted in the urine after administration of a single oral dose of 100 mg/kg to male Sprague-Dawley rats. Bowman *et al.* (1982) administered 12 mg/kg ^{14}C-labeled C.I. Direct Blue 15 or molar equivalent doses of 3,3′-dimethylbenzidine or 3,3′-dimethoxybenzidine to male F344/N rats by oral gavage. Approximately 19% of the dose was recovered in urine of animals treated with C.I. Direct Blue 15; 35% to 40% of the dose was recovered in urine of animals administered 3,3′-dimethylbenzidine or 3,3′dimethoxybenzidine. The doses of 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine, C.I. Direct Blue 15, or C.I. Acid Red 114 used in the NTP 2-year drinking water studies were higher than those used in the absorption studies of Bowman *et al.* (1982). The exact absorption cannot be extrapolated simply from the doses administered in the NTP studies. However, for the doses used in the NTP studies, 3,3′-dimethylbenzidine and C.I. Acid Red 114 appear to affect the liver more than do 3,3′-dimethoxybenzidine or C.I. Direct Blue 15. Such effects may be attributed to enhanced absorption or metabolism.

The benzidine dyes are metabolized to their parent congeners or to *N*-acetyl derivatives of their congeners and are excreted in urine (Lynn *et al.*, 1980, 1984; Bowman *et al.*, 1982; Nony *et al*, 1983; Rodgers *et al.*, 1983). Studies with benzidine have shown that the ultimate carcinogenic moiety is an activated form of benzidine produced via metabolic azo reduction. The sequence of benzidine metabolism is thought to begin first with *N*-acetylation followed by *N*-hydroxylation to form *N*′-hydroxy-*N*-acetylbenzidine which is further metabolized by glucuronidation. Prostaglandin H synthetase can activate hydroxylamines resulting in electrophilic

intermediates which bind to DNA (Wise *et al.*, 1984; Wang *et al.*, 1990).

Susceptibility of a species to the carcinogenic action of aromatic amines depends in part on the ability of the species to *N*-hydroxylate the amine substituent. *N*-Hydroxylation appears to be a necessary step in the metabolic activation of aromatic amines. *N*-Acyl and *N*-acetyl aromatic amine derivatives require activation to reactive esters, which act as ultimate carcinogens (Miller and Miller, 1977). Formation of various esters by different species may result in variations in organ specificity (Cohen, 1983). Although the Zymbal's gland has been reported to be deficient in sulfotransferase activity (Irving *et al.*, 1971) and transacylase activity (Bartsch *et al.*, 1973), it is capable of hydroxylating compounds via cytochrome P_{450}-dependent enzymatic pathways (Pohl and Fouts, 1983). In the case of these benzidine compounds, the Zymbal's gland is particularly susceptible to tumor formation.

Because preputial gland neoplasms usually are not overtly aggressive or invasive and rarely metastasize (Goodman *et al.*, 1979; Reznik and Ward, 1981), classification of these neoplasms as benign or malignant is difficult (Maronpot *et al.*, 1988). Studies by Ward and Lynch (1984) showed that malignant preputial/clitoral gland neoplasms from aging F344/N rats were transplantable at a higher incidence and with shorter latency periods than benign neoplasms. These conclusions were based on a single-passage study with a single carcinoma and four adenomas.

The transplantability of preputial gland neoplasms induced by 3,3′-dimethoxybenzidine dihydrochloride, C.I. Direct Blue 15, or C.I. Acid Red 114 in male F344/N rats was investigated to provide information on the biologic behavior of these neoplasms (Maronpot *et al.*, 1988; Ulland *et al.*, 1989). All neoplasms selected for transplanting were retrospectively diagnosed as carcinomas and therefore comparable information was not obtained for preputial gland adenomas. The transplanted preputial gland neoplasms did not become anaplastic or less differentiated over four serial passages. However, the transplants behaved biologically as malignant neoplasms in spite of their well-differentiated morphology. Transplants grew rapidly, reaching 3.0 cm in 7 to 9 weeks. No differences were observed in morphology or growth of transplants obtained from the controls or animals dosed with benzidine congener or dye. The results of these studies confirm the malignant nature of these preputial gland neoplasms from rats.

Tumor formation occurred at many sites after the administration of C.I. Acid Red 114. The mechanism for this tumor formation is thought to be due in part to genetic toxicity of the benzidine compounds. All of the chemicals tested positive in the *Salmonella* assay (Table 20). Cleavage of the azo bonds by reductive metabolism was necessary before positive results could be obtained for C.I. Acid Red 114 or C.I. Direct Blue 15 in the *Salmonella* tests. Tumors from animals treated with these benzidine compounds were shown to have activated *ras* genes (Reynolds *et al.*, 1990).

There is considerable evidence indicating that the activation of protooncogenes and the loss of specific regulatory substances, such as suppressor genes, may be distinct steps in the process of carcinogenesis (Barrett *et al.*, 1987). Activated oncogenes have been detected in only 3% of the spontaneous tumors of Fischer rats in contrast with the detection of activated H-*ras* or N-*ras* in 68% of epithelial tumors induced by benzidine congeners and derived dyes (Reynolds *et al.*, 1990). Furthermore, the presence of these activated oncogenes in several benign tumors suggests that *ras* activation may be an early event in the induction of neoplasms by these compounds. Thus, the activation of *ras* genes by point mutation is an important step in the induction of tumors, at least in rats, by this class of benzidine derived compounds.

TABLE 20
Comparison of National Toxicology Program Mutagenicity Test Results for Selected Benzidine Dyes[a]

Chemical Name	*Salmonella*[b]	CHO SCE	CHO Abs	*Drosophila* SLRL	*Drosophila* RT
3,3′-Dimethoxybenzidine	+	+	+	–	
3,3′-Dimethylbenzidine	+	+	+	+	–
C.I. Acid Red 114	+	–	–	–	
C.I. Direct Blue 218	–	+w	–	–	
C.I. Direct Blue 15	+	–	–		

[a] CHO SCE = Chinese hamster ovary cell sister chromatid exchange test; CHO Abs = Chinese hamster ovary cell chromosomal aberration test; SLRL = sex-linked recessive lethal test; RT = reciprocal translocation test; + = positive; – = negative; +w = weak evidence for positive response. For description of S9 source and details of experimental technique, see Appendix C. Only the *Salmonella* test was conducted with reductive metabolism.

[b] Positive responses were observed in *Salmonella typhimurium* strain TA1538 after incubation in a bacterial reduction system. Such a protocol allows for *in vitro* reduction of the azo linkages, mimicking the metabolism of these compounds in the human intestinal tract, and release of the parent amine, which can then be oxidatively metabolized using an induced rat or hamster liver S9 system (Reid *et al.*, 1984a). The first three chemicals were also positive when tested in a standard preincubation protocol.

CONCLUSIONS

Under the conditions of these 2-year drinking water studies, there was *clear evidence of carcinogenic activity** of C.I. Acid Red 114 for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, and liver. Increased incidences of neoplasms of the oral cavity epithelium, adrenal gland, and lung may have been related to chemical administration. There was *clear evidence of carcinogenic activity* for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity epithelium, small and large intestines, and lung. Increased incidences of mononuclear cell leukemia, mammary gland adenocarcinoma, and adrenal gland pheochromocytomas may have been related to chemical administration.

*Explanation of Levels of Evidence of Carcinogenic Activity appears on page 8. A summary of peer review comments and the public discussion on this Technical Report appears on page 10.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF C.I. ACID RED 114

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

	0 ppm	70 ppm	150 ppm	300 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
Interim evaluations	20	10	10	20
Early deaths				
Natural death	13	11	16	16
Moribund	13	9	23	33
Survivors				
Terminal sacrifice	23	15	26	1
Died last week of studies	1			
Animals examined microscopically	50	35	65	50
Alimentary System				
Intestine large, cecum	(50)	(35)	(64)	(49)
Intestine large, colon	(50)	(35)	(64)	(50)
Intestine small, ileum	(50)	(35)	(64)	(49)
Intestine small, jejunum	(50)	(35)	(64)	(49)
Adenocarcinoma, cystic, mucinous				1 (2%)
Leiomyoma		1 (3%)		
Liver	(50)	(35)	(65)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)			
Hepatocellular carcinoma		1 (3%)	5 (8%)	7 (14%)
Hepatocellular carcinoma, multiple			1 (2%)	
Neoplasm NOS, metastatic, uncertain primary site		1 (3%)		
Neoplastic nodule	2 (4%)		8 (12%)	12 (24%)
Neoplastic nodule, multiple		1 (3%)	2 (3%)	3 (6%)
Mesentery	(11)	(6)	(1)	(6)
Carcinoma, metastatic, islets, pancreatic	1 (9%)			
Pancreas	(50)	(35)	(64)	(49)
Pharynx			(1)	
Papilloma squamous			1 (100%)	
Salivary glands	(49)	(35)	(64)	(50)
Carcinoma	1 (2%)			
Stomach, glandular	(50)	(35)	(65)	(50)
Tongue				(3)
Papilloma squamous				2 (67%)
Cardiovascular System				
Heart	(50)	(35)	(65)	(50)
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Schwannoma NOS			1 (2%)	
Endocardium, schwannoma NOS			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Endocrine System				
Adrenal gland	(50)	(35)	(63)	(50)
Pheochromocytoma benign				1 (2%)
Adrenal gland, cortex	(50)	(35)	(63)	(50)
Bilateral, pheochromocytoma benign		1 (3%)		
Adrenal gland, medulla	(50)	(35)	(63)	(49)
Pheochromocytoma malignant	2 (4%)		3 (5%)	2 (4%)
Pheochromocytoma benign	15 (30%)	10 (29%)	19 (30%)	12 (24%)
Bilateral, pheochromocytoma benign	1 (2%)	1 (3%)	5 (8%)	7 (14%)
Islets, pancreatic	(50)	(35)	(65)	(50)
Adenoma	1 (2%)			
Carcinoma	1 (2%)	1 (3%)		
Pituitary gland	(50)	(35)	(65)	(47)
Carcinoma, metastatic, Zymbal's gland			1 (2%)	
Pars distalis, adenoma	12 (24%)	7 (20%)	14 (22%)	4 (9%)
Thyroid gland	(50)	(35)	(65)	(49)
C-cell, adenoma	5 (10%)	2 (6%)	6 (9%)	3 (6%)
C-cell, carcinoma	1 (2%)	1 (3%)		1 (2%)
Follicular cell, adenoma			1 (2%)	2 (4%)
Follicular cell, carcinoma	2 (4%)	2 (6%)	2 (3%)	
General Body System				
Tissue NOS	(1)		(3)	(3)
Carcinoma, metastatic, Zymbal's gland			1 (33%)	
Schwannoma malignant, metastatic, skin			1 (33%)	
Genital System				
Epididymis	(50)	(35)	(64)	(50)
Preputial gland	(44)	(34)	(60)	(48)
Adenoma	2 (5%)	1 (3%)	2 (3%)	1 (2%)
Carcinoma	4 (9%)	2 (6%)	3 (5%)	4 (8%)
Bilateral, carcinoma	1 (2%)	1 (3%)		
Prostate	(50)	(34)	(64)	(50)
Seminal vesicle	(49)	(34)	(65)	(48)
Testes	(50)	(35)	(64)	(50)
Bilateral, interstitial cell, adenoma	37 (74%)	24 (69%)	51 (80%)	38 (76%)
Interstitial cell, adenoma	7 (14%)	11 (31%)	8 (13%)	5 (10%)
Hematopoietic System				
Bone marrow	(50)	(35)	(64)	(50)
Lymph node	(49)	(35)	(65)	(50)
Lymph node, mandibular	(49)	(35)	(65)	(49)
Lymph node, mesenteric	(49)	(35)	(64)	(50)
Spleen	(50)	(35)	(64)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)			
Fibrosarcoma	1 (2%)			
Lipoma	1 (2%)			
Thymus	(41)	(22)	(47)	(40)
Neoplasm NOS		1 (5%)		
Sarcoma, metastatic, uncertain primary site				1 (3%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Integumentary System				
Mammary gland	(46)	(31)	(61)	(37)
Adenoma		1 (3%)		
Fibroadenoma	2 (4%)		3 (5%)	1 (3%)
Skin	(50)	(35)	(65)	(50)
Basal cell adenoma	1 (2%)	3 (9%)	12 (18%)	11 (22%)
Basal cell adenoma, multiple		1 (3%)	14 (22%)	19 (38%)
Basal cell carcinoma		1 (3%)	3 (5%)	5 (10%)
Basal cell carcinoma, multiple			2 (3%)	1 (2%)
Keratoacanthoma	1 (2%)	1 (3%)	4 (6%)	5 (10%)
Keratoacanthoma, multiple				2 (4%)
Papilloma squamous	1 (2%)		3 (5%)	2 (4%)
Sarcoma, metastatic, uncertain primary site				1 (2%)
Squamous cell carcinoma		2 (6%)	6 (9%)	6 (12%)
Squamous cell carcinoma, multiple			2 (3%)	1 (2%)
Sebaceous gland, adenoma		1 (3%)	4 (6%)	3 (6%)
Sebaceous gland, adenoma, multiple			1 (2%)	2 (4%)
Sebaceous gland, carcinoma	1 (2%)			1 (2%)
Subcutaneous tissue, chordoma, metastatic	1 (2%)			
Subcutaneous tissue, fibroma	4 (8%)	3 (9%)	5 (8%)	1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, lipoma				1 (2%)
Subcutaneous tissue, schwannoma malignant			1 (2%)	
Musculoskeletal System				
Bone				(2)
Sternum, hemangiosarcoma				1 (50%)
Skeletal muscle		(1)		(1)
Nervous System				
Brain	(50)	(35)	(64)	(50)
Astrocytoma malignant		1 (3%)		1 (2%)
Carcinoma, metastatic, Zymbal's gland			1 (2%)	
Glioma malignant			1 (2%)	
Peripheral nerve				(1)
Schwannoma malignant				1 (100%)
Respiratory System				
Lung	(50)	(35)	(65)	(50)
Alveolar/bronchiolar adenoma		2 (6%)	1 (2%)	3 (6%)
Alveolar/bronchiolar carcinoma	2 (4%)		1 (2%)	
Fibrosarcoma, metastatic, skin	1 (2%)			
Hepatocellular carcinoma, metastatic, liver				1 (2%)
Sarcoma, metastatic, uncertain primary site				1 (2%)
Squamous cell carcinoma, metastatic, skin		1 (3%)		2 (4%)
Nose	(50)	(35)	(65)	(50)
Squamous cell carcinoma				1 (2%)
Trachea	(50)	(35)	(65)	(50)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Special Senses System				
Eye		(1)	(2)	(1)
Zymbal's gland	(50)	(35)	(64)	(50)
Adenoma			1 (2%)	1 (2%)
Carcinoma			7 (11%)	6 (12%)
Schwannoma malignant, metastatic, skin			1 (2%)	
Urinary System				
Kidney	(50)	(35)	(65)	(50)
Squamous cell carcinoma, metastatic, skin				1 (2%)
Renal tubule, adenoma			1 (2%)	
Transitional epithelium, carcinoma	1 (2%)			
Urinary bladder	(50)	(34)	(64)	(50)
Systemic Lesions				
Multiple organs[a]	(50)	(35)	(65)	(50)
Leukemia mononuclear	20 (40%)	20 (57%)	37 (57%)	12 (24%)
Lymphoma malignant	1 (2%)			1 (2%)
Mesothelioma malignant	4 (8%)	6 (17%)	4 (6%)	3 (6%)
Tumor Summary				
Total animals with primary neoplasms[b]	49	35	64	49
Total primary neoplasms	135	110	246	196
Total animals with benign neoplasms	48	35	61	45
Total benign neoplasms	92	71	166	141
Total animals with malignant neoplasms	33	27	54	42
Total malignant neoplasms	43	38	78	55
Total animals with secondary neoplasms[c]	5	7	4	7
Total secondary neoplasms	14	23	7	18
Total animals with malignant neoplasms of uncertain primary site		1		1
Total animals with neoplasms uncertain-benign or malignant		1	2	
Total uncertain neoplasms		1	2	

[a] Number of animals with any tissue examined microscopically
[b] Primary tumors: all tumors except metastatic tumors
[c] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm

Number of Days on Study	1 3 4 4 4 5 5 5 5 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 3 5 5 5 7 2 6 6 9 0 0 3 4 5 5 7 8 8 8 9 0 0 1 1 1 1 5 4 8 5 9 4 9 0 7 7 6 1 0 1 1 0 2 7 6 1 6 1 5 5
Carcass ID Number	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 0 1 1 1 0 0 1 1 0 0 0 1 1 1 0 0 0 1 4 0 2 4 7 7 6 5 3 1 3 6 9 2 1 5 9 7 0 1 4 9 5 8 2 5 5 5 4 5 4 5 5 5 5 4 4 5 4 4 4 4 3 4 3 3 3 3 5 3
Alimentary System	
Esophagus	+ +
Intestine large	+ +
Intestine large, cecum	+ +
Intestine large, colon	+ +
Intestine large, rectum	+ +
Intestine small	+ +
Intestine small, duodenum	+ +
Intestine small, ileum	+ +
Intestine small, jejunum	+ +
Liver	+ +
Carcinoma, metastatic, islets, pancreatic	X
Neoplastic nodule	X
Mesentery	+ +
Carcinoma, metastatic, islets, pancreatic	X
Mesothelioma malignant, metastatic, testes	X
Pancreas	+ +
Salivary glands	+ + + + + + + + + + + + + + + + M + + + + + + + +
Carcinoma	
Stomach	+ +
Stomach, forestomach	+ +
Stomach, glandular	+ +
Tooth	+
Cardiovascular System	
Heart	+ +
Endocrine System	
Adrenal gland	+ +
Adrenal gland, cortex	+ +
Adrenal gland, medulla	+ +
Pheochromocytoma malignant	X X
Pheochromocytoma benign	X X X X X X
Bilateral, pheochromocytoma benign	X
Islets, pancreatic	+ +
Adenoma	
Carcinoma	X

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm (continued)

Number of Days on Study	7 2 9	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 1 0 1	0 0 5 1	0 0 5 2	0 0 6 1	0 0 6 2	0 0 6 3	0 0 7 1	0 0 7 2	0 0 8 1	0 0 8 2	0 0 8 3	0 0 8 4	0 0 9 1	0 0 9 2	0 0 9 2	0 1 0 3	0 1 0 3	0 1 1 1	0 1 1 2	0 1 1 2	0 1 2 1	0 1 2 2	0 1 3 1	0 1 4 1	0 1 3 2	0 1 3 3	0 1 4 2	**Total Tissues/ Tumors**
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, islets, pancreatic																											1
Neoplastic nodule						X																					2
Mesentery			+																						+		11
Carcinoma, metastatic, islets, pancreatic																											1
Mesothelioma malignant, metastatic, testes																											1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma														X													1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																											1
Cardiovascular System																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																											2
Pheochromocytoma benign		X	X		X	X			X									X			X		X	X	X		15
Bilateral, pheochromocytoma benign																											1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																									X		1
Carcinoma																											1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm (continued)

Number of Days on Study	1 3 4 4 4 5 5 5 5 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 3 5 5 5 7 2 6 6 9 0 0 3 4 5 5 7 8 8 8 9 0 0 1 1 1 1 5 4 8 5 9 4 9 0 7 7 6 1 0 1 1 0 2 7 6 1 6 1 5 5
Carcass ID Number	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 0 1 1 1 0 0 1 1 0 0 0 1 1 1 0 0 0 1 4 0 2 4 7 7 6 5 3 1 3 6 9 2 1 5 9 7 0 1 4 9 5 8 2 5 5 5 4 5 4 5 5 5 5 4 4 5 4 4 4 4 3 4 3 3 3 3 5 3
Endocrine System (continued)	
Parathyroid gland	+ M +
Pituitary gland	+ +
Pars distalis, adenoma	X X X X X X X X X X
Thyroid gland	+ +
C-cell, adenoma	X X X X
C-cell, carcinoma	
Follicular cell, carcinoma	X
General Body System	
Tissue NOS	
Genital System	
Epididymis	+ +
Mesothelioma malignant, metastatic, testes	X X X X
Preputial gland	+ +
Adenoma	
Carcinoma	X
Bilateral, carcinoma	X
Prostate	+ +
Seminal vesicle	M +
Mesothelioma malignant, metastatic, testes	X X
Testes	+ +
Bilateral, interstitial cell, adenoma	X X X X X X X X X X X X X X
Interstitial cell, adenoma	X X X X X X X
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ + + + + + + + + + + + + + + + + M + + + + + + +
Lymph node, mandibular	+ + + + + + + + + + + + + + + + + M + + + + + + +
Lymph node, mesenteric	+ + + + + + + + + + + + + + + + + M + + + + + + +
Spleen	+ +
Carcinoma, metastatic, islets, pancreatic	X
Fibrosarcoma	X
Lipoma	
Mesothelioma malignant, metastatic, testes	X
Thymus	+ + + + M + + + + + M + + + + + + + M + + + + + +

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm (continued)

Number of Days on Study	729	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	733	733	
Carcass ID Number	00101	00011	00052	00061	00062	00063	00071	00072	00081	00082	00083	00084	00091	00092	00093	00111	00112	00113	00121	00122	00123	00124	00131	00132	00133	00134	**Total Tissues/ Tumors**
Endocrine System (continued)																											
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X					X					X						X									12
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma											X							X									5
C-cell, carcinoma	X																										1
Follicular cell, carcinoma																				X							2
General Body System																											
Tissue NOS																	+										1
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, testes																											3
Preputial gland	+	+	+	+	M	+	+	+	M	M	+	+	+	+	+	+	M	M	+	+	+	+	M	+	+		44
Adenoma													X			X											2
Carcinoma							X													X				X			4
Bilateral, carcinoma																											1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Mesothelioma malignant, metastatic, testes																											2
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	37
Interstitial cell, adenoma																		X									7
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, islets, pancreatic																											1
Fibrosarcoma																											1
Lipoma																									X		1
Mesothelioma malignant, metastatic, testes																											1
Thymus	+	M	+	+	+	M	+	+	+	+	+	+	M	+	+	+	M	M	+	+	M	+	+	+	+		41

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm (continued)

Number of Days on Study	1 3 4 4 4 5 5 5 5 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 3 5 5 5 7 2 6 6 9 0 0 3 4 5 5 7 8 8 8 9 0 0 1 1 1 1 5 4 8 5 9 4 9 0 7 7 6 1 0 1 1 0 2 7 6 1 6 1 5 5
Carcass ID Number	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 0 1 1 1 0 0 1 1 0 0 0 1 1 1 0 0 0 1 4 0 2 4 7 7 6 5 3 1 3 6 9 2 1 5 9 7 0 1 4 9 5 8 2 5 5 5 4 5 4 5 5 5 5 4 4 5 4 4 4 4 3 4 3 3 3 3 5 3
Integumentary System	
Mammary gland	+ M M + M +
Fibroadenoma	
Skin	+ +
Basal cell adenoma	
Keratoacanthoma	
Papilloma squamous	
Sebaceous gland, carcinoma	X
Subcutaneous tissue, chordoma, metastatic	
Subcutaneous tissue, fibroma	X
Subcutaneous tissue, fibrosarcoma	X
Musculoskeletal System	
None	
Nervous System	
Brain	+ +
Respiratory System	
Lung	+ +
Alveolar/bronchiolar carcinoma	
Fibrosarcoma, metastatic, skin	X
Nose	+ +
Trachea	+ +
Special Senses System	
Zymbal's gland	+ +
Urinary System	
Kidney	+ +
Transitional epithelium, carcinoma	X
Urinary bladder	+ +
Mesothelioma malignant, metastatic, testes	X X
Systemic Lesions	
Multiple organs	+ +
Leukemia mononuclear	X X X X X X X X X X
Lymphoma malignant	X
Mesothelioma malignant	X X X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm (continued)

Number of Days on Study	729	732	732	732	732	732	733	732	732	732	732	732	733	732	732	732	732	732	732	732	733	733	733	733	733	
Carcass ID Number	01001	01005	01012	01021	01026	01036	01071	01072	01081	01082	01083	01084	01091	01092	01102	01103	01111	01121	01122	01201	01202	01203	01204	01211	01213	01221	**Total Tissues/ Tumors**
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	46
Fibroadenoma			X	X																							2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma													X														1
Keratoacanthoma																X											1
Papilloma squamous														X													1
Sebaceous gland, carcinoma																											1
Subcutaneous tissue, chordoma, metastatic																			X								1
Subcutaneous tissue, fibroma							X		X																X		4
Subcutaneous tissue, fibrosarcoma																											1
Musculoskeletal System																											
None																											
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																			X	X							2
Fibrosarcoma, metastatic, skin																											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																											
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, carcinoma																											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, testes																											2
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X		X	X			X			X		X	X								X	X			20
Lymphoma malignant																											1
Mesothelioma malignant																		X									4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 70 ppm

Number of Days on Study	3 5 2	4 4 7	4 6 5	5 2 4	5 3 0	5 3 0	5 4 5	6 3 1	6 4 1	6 4 9	6 5 1	6 5 2	6 5 8	6 6 6	6 9 4	6 9 4	6 9 4	7 0 1	7 0 5	7 2 4	7 3 2	7 3 2	7 3 2	7 3 2
Carcass ID Number	0 3 4 5	0 3 3 5	0 3 6 5	0 3 4 4	0 3 2 5	0 3 6 4	0 3 5 5	0 3 1 5	0 3 1 4	0 3 2 4	0 3 7 5	0 3 7 4	0 3 2 3	0 3 5 4	0 3 3 4	0 3 4 3	0 3 5 3	0 3 5 2	0 3 6 3	0 3 7 3	0 3 1 1	0 3 1 2	0 3 1 3	0 3 2 1	0 3 2 2
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																								
Mesothelioma malignant, metastatic, testes																							X	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																	X							
Mesothelioma malignant, metastatic, testes			X																				X	
Neoplasm nos, metastatic, uncertain primary site																				X				
Neoplastic nodule, multiple																								
Mesentery		+	+														+						+	
Mesothelioma malignant, metastatic, testes		X	X																				X	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes			X																				X	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, pheochromocytoma benign																X								
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign				X													X				X			X
Bilateral, pheochromocytoma benign																X								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 70 ppm (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 3 3 1	0 3 3 2	0 3 3 3	0 3 4 1	0 3 4 2	0 3 5 1	0 3 6 1	0 3 6 2	0 3 7 1	0 3 7 2	**Total Tissues/ Tumors**
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	35
Intestine large	+	+	+	+	+	+	+	+	+	+	35
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	35
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	35
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	34
Intestine small	+	+	+	+	+	+	+	+	+	+	35
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	35
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	35
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	35
Leiomyoma		X									1
Mesothelioma malignant, metastatic, testes											1
Liver	+	+	+	+	+	+	+	+	+	+	35
Hepatocellular carcinoma											1
Mesothelioma malignant, metastatic, testes											2
Neoplasm nos, metastatic, uncertain primary site											1
Neoplastic nodule, multiple										X	1
Mesentery	+		+								6
Mesothelioma malignant, metastatic, testes											3
Pancreas	+	+	+	+	+	+	+	+	+	+	35
Mesothelioma malignant, metastatic, testes											2
Salivary glands	+	+	+	+	+	+	+	+	+	+	35
Stomach	+	+	+	+	+	+	+	+	+	+	35
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	35
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	35
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	35
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	35
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	35
Bilateral, pheochromocytoma benign											1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	35
Pheochromocytoma benign		X				X	X	X	X	X	10
Bilateral, pheochromocytoma benign											1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 70 ppm (continued)

Number of Days on Study	345	446	452	554	553	563	574	663	664	674	695	706	716	769	799	799	801	870	872	873	873	873	873
Carcass ID Number	00334	00336	00345	00344	00353	00363	00355	00331	00341	00342	00337	00347	00332	00335	00333	00344	00345	00355	00366	00371	00381	00393	00392
Endocrine System (continued)																							
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma													X										
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X						X			X			X				X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																							
C-cell, carcinoma													X										
Follicular cell, carcinoma										X													X
General Body System																							
None																							
Genital System																							
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes		X	X										X			X						X	
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Adenoma																							
Carcinoma				X	X																		
Bilateral, carcinoma																							
Prostate	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes		X																				X	
Seminal vesicle	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes			X																			X	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma					X	X		X	X	X		X	X			X	X	X	X	X	X	X	X
Interstitial cell, adenoma	X	X	X	X			X				X			X	X						X		
Hematopoietic System																							
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes			X																				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 70 ppm (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 3 3 1	0 3 3 2	0 3 3 3	0 3 4 1	0 3 4 2	0 3 5 1	0 3 6 1	0 3 6 2	0 3 7 1	0 3 7 2	**Total Tissues/ Tumors**
Endocrine System (continued)											
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	35
Carcinoma											1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	35
Pituitary gland	+	+	+	+	+	+	+	+	+	+	35
Pars distalis, adenoma		X					X				7
Thyroid gland	+	+	+	+	+	+	+	+	+	+	35
C-cell, adenoma		X						X			2
C-cell, carcinoma											1
Follicular cell, carcinoma											2
General Body System											
None											
Genital System											
Epididymis	+	+	+	+	+	+	+	+	+	+	35
Mesothelioma malignant, metastatic, testes											5
Preputial gland	+	+	+	+	+	+	+	+	+	+	34
Adenoma					X						1
Carcinoma											2
Bilateral, carcinoma							X				1
Prostate	+	+	+	+	+	+	+	+	+	+	34
Mesothelioma malignant, metastatic, testes											2
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	34
Mesothelioma malignant, metastatic, testes											2
Testes	+	+	+	+	+	+	+	+	+	+	35
Bilateral, interstitial cell, adenoma	X	X	X		X	X	X	X	X		24
Interstitial cell, adenoma				X						X	11
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	35
Lymph node	+	+	+	+	+	+	+	+	+	+	35
Mesothelioma malignant, metastatic, testes											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	35

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 70 ppm (continued)

Number of Days on Study	3 5 2	4 4 7	4 6 5	5 2 4	5 3 0	5 3 0	5 4 5	6 3 1	6 4 1	6 4 9	6 5 1	6 5 2	6 5 8	6 6 6	6 9 4	6 9 4	6 9 4	7 0 1	7 0 5	7 2 4	7 3 2	7 3 2	7 3 2	7 3 2
Carcass ID Number	0 3 4 5	0 3 3 5	0 3 6 5	0 3 4 4	0 3 2 5	0 3 6 4	0 3 5 5	0 3 1 5	0 3 1 4	0 3 2 4	0 3 7 5	0 3 7 4	0 3 2 3	0 3 5 4	0 3 3 4	0 3 4 3	0 3 5 3	0 3 5 2	0 3 6 3	0 3 7 3	0 3 1 1	0 3 1 2	0 3 1 3	0 3 2 1	0 3 2 2
Hematopoietic System (continued)																								
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes		X																						
Thymus	+	+	+	M	+	+	+	+	M	+	M	M	M	+	+	+	+	+	+	+	M	M	+	M	M
Neoplasm NOS								X																
Integumentary System																								
Mammary gland	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+
Adenoma																								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma											X													
Basal cell adenoma, multiple																								X
Basal cell carcinoma																				X				
Keratoacanthoma																								
Squamous cell carcinoma																								
Sebaceous gland, adenoma																								
Subcutaneous tissue, fibroma								X	X				X											
Musculoskeletal System																								
Skeletal muscle	+																							
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																					X			
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																		X						
Squamous cell carcinoma, metastatic, skin																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Eye	+																							
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 70 ppm (continued)

Number of Days on Study	7 7 7 7 7 7 7 7 7 7 3 3 3 3 3 3 3 3 3 3 2 2 2 2 2 2 2 2 2 2	
Carcass ID Number	0 0 0 0 0 0 0 0 0 0 3 3 3 3 3 3 3 3 3 3 3 3 3 4 4 5 6 6 7 7 1 2 3 1 2 1 1 2 1 2	**Total Tissues/ Tumors**
Hematopoietic System (continued)		
Lymph node, mesenteric	+ + + + + + + + + +	35
Spleen	+ + + + + + + + + +	35
Mesothelioma malignant, metastatic, testes		1
Thymus	M + + + + + M M M +	22
Neoplasm NOS		1
Integumentary System		
Mammary gland	+ + + + + + + + + +	31
Adenoma	X	1
Skin	+ + + + + + + + + +	35
Basal cell adenoma	X X	3
Basal cell adenoma, multiple		1
Basal cell carcinoma		1
Keratoacanthoma	X	1
Squamous cell carcinoma	X X	2
Sebaceous gland, adenoma	X	1
Subcutaneous tissue, fibroma		3
Musculoskeletal System		
Skeletal muscle		1
Nervous System		
Brain	+ + + + + + + + + +	35
Astrocytoma malignant		1
Respiratory System		
Lung	+ + + + + + + + + +	35
Alveolar/bronchiolar adenoma	X	2
Squamous cell carcinoma, metastatic, skin	X	1
Nose	+ + + + + + + + + +	35
Trachea	+ + + + + + + + + +	35
Special Senses System		
Eye		1
Zymbal's gland	+ + + + + + + + + +	35

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 70 ppm (continued)

Number of Days on Study	3	4	4	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7
	5	4	6	2	3	3	4	3	4	4	5	5	5	6	9	9	9	0	0	2	3	3	3	3
	2	7	5	4	0	0	5	1	1	9	1	2	8	6	4	4	4	1	5	4	2	2	2	2
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	4	3	6	4	2	6	5	1	1	2	7	7	2	5	3	4	5	5	6	7	1	1	2	2
	5	5	5	4	5	4	5	5	4	4	5	4	3	4	4	3	3	2	3	3	1	2	1	2
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes		X	X																					
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X			X			X			X			X	X	X	X	X	X	X	X	X	X	X	X
Mesothelioma malignant		X	X										X			X							X	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 70 ppm (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 3 3 1	0 3 3 2	0 3 3 3	0 3 4 1	0 3 4 2	0 3 5 1	0 3 6 1	0 3 6 2	0 3 7 1	0 3 7 2	**Total Tissues/ Tumors**
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	35
Urinary bladder	+	+	+	+	+	+	+	+	+	+	34
Mesothelioma malignant, metastatic, testes											2
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear					X				X	X	20
Mesothelioma malignant											6

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm

Number of Days on Study	3 2 5	4 0 3	4 5 5	4 9 9	5 3 0	5 6 5	5 6 7	5 9 3	5 9 4	5 9 9	6 1 4	6 3 8	6 4 1	6 4 1	6 4 1	6 4 1	6 4 1	6 5 4	6 5 4	6 5 7	6 6 3	6 6 6	6 6 9	6 7 2	6 7 3
Carcass ID Number	0 4 9 5	0 5 1 5	0 5 6 5	0 6 1 5	0 5 4 5	0 6 0 5	0 6 1 4	0 5 4 4	0 6 0 4	0 5 1 4	0 5 3 5	0 5 3 4	0 5 4 4	0 4 9 4	0 5 2 4	0 5 2 5	0 5 7 5	0 5 9 5	0 5 6 4	0 5 8 5	0 5 1 3	0 5 4 3	0 5 7 4	0 5 2 3	0 5 9 4	0 6 1 3
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular carcinoma, multiple																									
Neoplastic nodule																X									
Neoplastic nodule, multiple																						X			
Mesentery																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx																									
Papilloma squamous																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma NOS																									
Endocardium, schwannoma NOS																									
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+
Pheochromocytoma malignant																									
Pheochromocytoma benign						X															X	X			X
Bilateral, pheochromocytoma benign																X									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland	X																								
Pars distalis, adenoma							X								X	X									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma					X																				
Follicular cell, adenoma																									
Follicular cell, carcinoma						X			X																

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	6 7 7	6 8 1	6 8 7	6 9 1	6 9 1	6 9 3	6 9 3	6 9 4	6 9 4	6 9 4	7 0 5	7 1 1	7 1 4	7 1 5	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2
Carcass ID Number	0 5 2 2	0 5 5	0 5 3 3	0 5 8 4	0 6 0 3	0 5 2 1	0 5 7 3	0 5 4 2	0 5 5 5	0 5 8 3	0 5 5 4	0 5 9 3	0 5 0 4	0 4 9 3	0 4 9 1	0 4 9 2	0 5 0 1	0 5 0 2	0 5 0 3	0 5 1 1	0 5 1 2	0 5 3 1	0 5 3 2	0 5 4 1	0 5 5 1
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma			X																					
Hepatocellular carcinoma, multiple																								
Neoplastic nodule							X								X									
Neoplastic nodule, multiple																								
Mesentery																								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Pharynx																				+				
Papilloma squamous																				X				
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma NOS																								
Endocardium, schwannoma NOS		X																						
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant					X													X						
Pheochromocytoma benign	X	X		X		X				X				X						X		X		
Bilateral, pheochromocytoma benign			X																				X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland																								
Pars distalis, adenoma	X		X					X	X												X		X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma						X		X													X			
Follicular cell, adenoma																				X				
Follicular cell, carcinoma																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 5 5 2	0 5 5 3	0 5 6 1	0 5 6 2	0 5 6 3	0 5 7 1	0 5 7 2	0 5 8 1	0 5 8 2	0 5 9 1	0 5 9 2	0 6 0 1	0 6 0 2	0 6 1 1	0 6 1 2	**Total Tissues/ Tumors**
Alimentary System																
Esophagus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Hepatocellular carcinoma		X		X		X		X								5
Hepatocellular carcinoma, multiple												X				1
Neoplastic nodule					X						X	X		X	X	8
Neoplastic nodule, multiple							X									2
Mesentery												+				1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Pharynx																1
Papilloma squamous																1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Schwannoma NOS													X			1
Endocardium, schwannoma NOS																1
Endocrine System																
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Pheochromocytoma malignant										X						3
Pheochromocytoma benign				X	X	X	X		X		X		X			19
Bilateral, pheochromocytoma benign												X		X		5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Carcinoma, metastatic, Zymbal's gland																1
Pars distalis, adenoma		X						X		X				X		14
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
C-cell, adenoma											X		X			6
Follicular cell, adenoma																1
Follicular cell, carcinoma																2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	3 2 5	4 0 3	4 5 5	4 9 9	5 3 0	5 6 5	5 6 7	5 9 3	5 9 4	5 9 9	5 9 4	6 1 8	6 3 1	6 4 1	6 4 1	6 4 1	6 4 1	6 4 1	6 5 4	6 5 4	6 5 7	6 6 3	6 6 6	6 6 9	6 7 2	6 7 3
Carcass ID Number	0 4 9 5	0 5 1 5	0 5 6 5	0 6 1 5	0 5 4 5	0 6 0 5	0 6 1 4	0 5 4 4	0 6 0 4	0 5 6 4	0 5 1 4	0 5 3 5	0 5 3 4	0 4 9 4	0 5 2 4	0 5 2 5	0 5 7 5	0 5 9 5	0 5 6 4	0 5 8 5	0 5 1 3	0 5 4 3	0 5 7 4	0 5 2 3	0 5 9 4	0 5 1 3
General Body System																										
Tissue NOS	+																					+				
Carcinoma, metastatic, Zymbal's gland	X																									
Schwannoma malignant, metastatic, skin																						X				
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes																										
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma						X																				
Carcinoma					X					X														X		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma					X	X		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma				X							X															
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	M	+	+	+	M	+	+	+	+	M	+	+	+	+	M	+	M	M	M	+	+	+	+	+
Integumentary System																										
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Fibroadenoma																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																X							X			
Basal cell adenoma, multiple																										
Basal cell carcinoma																										
Basal cell carcinoma, multiple																										X
Keratoacanthoma																										
Papilloma squamous																			X							X
Squamous cell carcinoma						X																				
Squamous cell carcinoma, multiple																										
Sebaceous gland, adenoma																										
Sebaceous gland, adenoma, multiple								X																		
Subcutaneous tissue, fibroma														X												
Subcutaneous tissue, schwannoma malignant																						X				

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	6 7 7	6 8 1	6 8 7	6 9 1	6 9 1	6 9 3	6 9 3	6 9 4	6 9 4	6 9 4	7 0 5	7 1 1	7 1 4	7 1 5	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2
Carcass ID Number	0 5 2 2	0 5 0 5	0 5 3 3	0 5 8 4	0 6 0 3	0 5 2 1	0 5 7 3	0 5 4 2	0 5 5 5	0 5 8 3	0 5 5 4	0 5 9 3	0 5 0 4	0 4 9 3	0 4 9 1	0 4 9 2	0 5 1 1	0 5 1 2	0 5 0 3	0 5 1 1	0 5 1 2	0 5 3 1	0 5 3 2	0 5 4 1	0 5 5 1
General Body System																								
Tissue NOS																								
Carcinoma, metastatic, Zymbal's gland																								
Schwannoma malignant, metastatic, skin																								
Genital System																								
Epididymis	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes							X	X																
Preputial gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Adenoma									X															
Carcinoma																								
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma	X		X		X	X	X	X	X			X			X	X	X	X	X		X	X	X	X	X
Interstitial cell, adenoma		X		X						X			X	X						X				
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	+	+	+	M	+	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+	M
Integumentary System																								
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Fibroadenoma																								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma	X																			X		X		X
Basal cell adenoma, multiple		X	X	X		X									X		X	X			X		X	
Basal cell carcinoma																							X	
Basal cell carcinoma, multiple																								
Keratoacanthoma																X							X	
Papilloma squamous																								
Squamous cell carcinoma					X				X														X	
Squamous cell carcinoma, multiple																								
Sebaceous gland, adenoma															X									
Sebaceous gland, adenoma, multiple																								
Subcutaneous tissue, fibroma							X						X						X					
Subcutaneous tissue, schwannoma malignant																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	
Carcass ID Number	0 5 5 2	0 5 5 3	0 5 6 1	0 5 6 2	0 5 6 3	0 5 7 1	0 5 7 2	0 5 8 1	0 5 8 2	0 5 9 1	0 5 9 2	0 5 0 1	0 6 0 2	0 6 1 1	0 6 1 2	0 6 1 2	**Total Tissues/ Tumors**
General Body System																	
Tissue NOS						+											3
Carcinoma, metastatic, Zymbal's gland																	1
Schwannoma malignant, metastatic, skin																	1
Genital System																	
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Mesothelioma malignant, metastatic, testes																	2
Preputial gland	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+		60
Adenoma																	2
Carcinoma																	3
Prostate	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+		64
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		64
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X		X		51
Interstitial cell, adenoma																	8
Hematopoietic System																	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		64
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		64
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		64
Thymus	+	M	M	+	M	+	+	+	+	+	+	+	M	M	+		47
Integumentary System																	
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		61
Fibroadenoma			X		X			X									3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Basal cell adenoma		X			X			X	X		X		X				12
Basal cell adenoma, multiple				X		X	X							X	X		14
Basal cell carcinoma											X			X			3
Basal cell carcinoma, multiple												X					2
Keratoacanthoma					X	X											4
Papilloma squamous													X				3
Squamous cell carcinoma								X				X					6
Squamous cell carcinoma, multiple											X				X		2
Sebaceous gland, adenoma						X						X		X			4
Sebaceous gland, adenoma, multiple																	1
Subcutaneous tissue, fibroma		X															5
Subcutaneous tissue, schwannoma malignant																	1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	3	4	4	4	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6
	2	0	5	9	3	6	6	9	9	9	1	3	4	4	4	4	4	5	5	5	6	6	6	7	7
	5	3	5	9	0	5	7	3	4	9	4	8	1	1	1	1	1	4	4	7	3	6	9	2	3
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	5	5	6	5	6	6	5	6	5	5	5	4	5	5	5	5	5	5	5	5	5	5	5	6
	9	1	6	1	4	0	1	4	0	1	3	3	9	2	2	7	9	6	8	1	4	7	2	9	1
	5	5	5	5	5	5	4	4	4	4	5	4	4	4	5	5	5	4	5	3	3	4	3	4	3
Musculoskeletal System																									
None																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland	X																								
Glioma malignant							X																		
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma														X											
Carcinoma	X	X															X						X		
Schwannoma malignant, metastatic, skin																					X				
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X	X				X	X	X	X	X	X		X	X	X	X	X	X		X			
Mesothelioma malignant																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	6 7 7	6 8 1	6 8 7	6 9 1	6 9 1	6 9 3	6 9 3	6 9 4	6 9 4	6 9 4	7 0 5	7 1 1	7 1 4	7 1 5	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2
Carcass ID Number	0 5 2 2	0 5 0 5	0 5 3 3	0 5 8 4	0 6 0 3	0 5 2 1	0 5 7 3	0 5 4 2	0 5 5 5	0 5 8 3	0 5 5 4	0 5 9 3	0 5 0 4	0 4 9 3	0 4 9 1	0 4 9 2	0 5 0 1	0 5 0 2	0 5 0 3	0 5 1 1	0 5 1 2	0 5 3 1	0 5 3 2	0 5 4 1	0 5 5 1
Musculoskeletal System																									
None																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland																									
Glioma malignant																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye													+												
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma				X																	X				
Schwannoma malignant, metastatic, skin																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X	X	X	X		X		X	X		X	X	X	X		X			X	X		X		
Mesothelioma malignant							X	X																	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3
Carcass ID Number	0 5 5 2	0 5 5 3	0 5 6 1	0 5 6 2	0 5 6 3	0 5 7 1	0 5 7 2	0 5 8 1	0 5 8 2	0 5 9 1	0 5 9 2	0 6 0 1	0 6 0 2	0 6 1 1	0 6 1 2	**Total Tissues/ Tumors**
Musculoskeletal System																
None																
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Carcinoma, metastatic, zymbal's gland																1
Glioma malignant																1
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Alveolar/bronchiolar adenoma																1
Alveolar/bronchiolar carcinoma			X													1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Special Senses System																
Eye										+						2
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	64
Adenoma																1
Carcinoma							X									7
Schwannoma malignant, metastatic, skin																1
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Renal tubule, adenoma															X	1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear					X			X			X	X	X		X	37
Mesothelioma malignant				X											X	4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm

Number of Days on Study	2 9 8	3 7 7	3 8 3	4 1 2	4 5 6	4 7 0	4 7 3	5 0 1	5 1 1	5 1 2	5 2 4	5 2 4	5 3 0	5 3 0	5 3 0	5 3 1	5 3 1	5 3 1	5 3 3	5 4 5	5 5 6	5 8 1	5 8 2	6 1 4	6 1 7
Carcass ID Number	0 7 9 5	0 8 8 5	0 8 2 5	0 8 0 5	0 8 5 5	0 8 6 4	0 8 6 5	0 8 6 3	0 8 5 4	0 8 1 3	0 8 2 4	0 8 3 5	0 8 4 5	0 8 4 4	0 8 8 5	0 8 9 3	0 8 5 5	0 9 7 5	0 9 0 5	0 9 9 4	0 8 5 2	0 8 4 4	0 9 0 4	0 8 6 2	0 8 8 1	0 8 3 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes								X																	
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes								X																	
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, cystic, mucinous																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma													X			X									
Neoplastic nodule																	X			X			X		X
Neoplastic nodule, multiple																									
Mesentery						+	+	+																	
Mesothelioma malignant, metastatic, testes								X																	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue								+																	
Papilloma squamous																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver																									
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pheochromocytoma malignant				X																					
Pheochromocytoma benign												X						X		X					
Bilateral, pheochromocytoma benign																								X	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	6 2 2	6 2 5	6 3 4	6 3 8	6 3 8	6 4 9	6 5 1	6 5 8	6 6 1	6 6 3	6 6 3	6 6 3	6 6 3	6 7 7	6 9 3	6 9 3	6 9 4	6 9 4	6 9 4	7 0 4	7 1 1	7 1 1	7 2 3	7 2 4	7 3 2	
Carcass ID Number	0 9 0 3	0 8 7 4	0 8 8 3	0 8 2 3	0 8 9 3	0 8 2 2	0 8 3 3	0 8 7 3	0 8 5 1	0 8 1 2	0 8 4 3	0 8 8 2	0 8 9 2	0 8 7 2	0 8 2 1	0 8 9 1	0 8 3 2	0 8 8 1	0 8 0 2	0 9 4 2	0 8 1 1	0 8 0 1	0 9 4 1	0 8 7 1	0 8 3 1	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	49
Mesothelioma malignant, metastatic, testes																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	49
Mesothelioma malignant, metastatic, testes																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	49
Adenocarcinoma, cystic, mucinous			X																							1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma			X	X	X					X									X							7
Neoplastic nodule			X		X						X						X	X		X		X		X		12
Neoplastic nodule, multiple															X						X				X	3
Mesentery			+																+		+					6
Mesothelioma malignant, metastatic, testes																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue											+														+	3
Papilloma squamous											X														X	2
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic, liver																			X							1
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																									X	1
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant	X																									2
Pheochromocytoma benign			X	X	X											X		X		X		X	X	X		12
Bilateral, pheochromocytoma benign										X	X	X			X		X				X					7

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	2 9 8	3 7 7	3 8 3	4 1 2	4 5 6	4 7 0	4 7 3	5 0 1	5 1 1	5 1 2	5 2 4	5 2 4	5 3 0	5 3 0	5 3 0	5 3 1	5 3 1	5 3 1	5 3 8	5 4 5	5 5 6	5 8 1	5 8 2	6 1 4	6 1 7
Carcass ID Number	0 7 9 5	0 8 8 5	0 8 2 5	0 8 0 5	0 8 5 5	0 8 6 4	0 8 6 5	0 8 6 3	0 8 5 4	0 8 1 3	0 8 2 4	0 8 3 5	0 8 4 5	0 8 8 4	0 8 9 5	0 8 5 3	0 8 7 5	0 9 0 5	0 9 5 4	0 8 5 2	0 8 4 4	0 9 0 4	0 8 6 2	0 8 6 1	0 8 3 4
Endocrine System (continued)																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X		X														X		X				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
C-cell, carcinoma																									
Follicular cell, adenoma											X														
General Body System																									
Tissue NOS																+	+								
Mesothelioma malignant, metastatic, testes																X	X								
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes																	X								
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X																				
Carcinoma													X	X	X										
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Mesothelioma malignant, metastatic, testes								X																	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma					X					X	X	X	X	X	X	X	X	X			X	X		X	X
Interstitial cell, adenoma						X													X	X			X		
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes								X																	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	M	M	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	M	+	+
Sarcoma, metastatic, uncertain primary site																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	6 2 2	6 2 5	6 3 4	6 3 8	6 3 8	6 4 9	6 5 1	6 5 8	6 6 1	6 6 3	6 6 3	6 6 3	6 6 3	6 7 7	6 9 3	6 9 3	6 9 4	6 9 4	6 9 4	7 0 4	7 1 1	7 1 1	7 2 3	7 2 4	7 3 2	
Carcass ID Number	0 9 0 3	0 8 7 4	0 8 8 3	0 8 2 3	0 8 9 3	0 8 2 2	0 8 3 3	0 8 7 3	0 8 5 1	0 8 1 2	0 8 4 3	0 8 8 2	0 8 9 2	0 8 7 2	0 8 2 1	0 8 9 1	0 8 3 2	0 8 8 1	0 8 0 2	0 9 4 2	0 8 1 1	0 9 0 1	0 8 4 1	0 8 7 1	0 8 3 1	**Total Tissues/ Tumors**
Endocrine System (continued)																										
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	M	+	+	47
Pars distalis, adenoma																										4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	49
C-cell, adenoma				X							X							X								3
C-cell, carcinoma													X													1
Follicular cell, adenoma																									X	2
General Body System																										
Tissue NOS																			+							3
Mesothelioma malignant, metastatic, testes																										2
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, testes																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	48
Adenoma																										1
Carcinoma																					X					4
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Mesothelioma malignant, metastatic, testes																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	38
Interstitial cell, adenoma		X																								5
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, testes																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	M	40
Sarcoma, metastatic, uncertain primary site								X																		1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	2 9 8	3 7 7	3 8 3	4 1 2	4 5 6	4 7 0	4 7 3	5 0 1	5 1 1	5 1 2	5 2 4	5 2 4	5 3 0	5 3 0	5 3 0	5 3 1	5 3 1	5 3 1	5 3 8	5 4 5	5 5 6	5 8 1	5 8 2	6 1 4	6 1 7
Carcass ID Number	0 7 9 5	0 8 8 5	0 8 2 5	0 8 0 5	0 8 5 5	0 8 6 4	0 8 6 5	0 8 6 3	0 8 5 4	0 8 1 3	0 8 2 4	0 8 3 5	0 8 4 5	0 8 8 4	0 8 9 5	0 8 5 3	0 8 7 5	0 9 0 5	0 8 9 4	0 8 5 2	0 8 4 4	0 9 0 4	0 8 6 2	0 8 6 1	0 8 3 4
Integumentary System																									
Mammary gland	+	M	+	M	+	M	+	M	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma							X				X		X	X											
Basal cell adenoma, multiple																X		X	X					X	X
Basal cell carcinoma							X													X		X			
Basal cell carcinoma, multiple																									
Keratoacanthoma																							X	X	
Keratoacanthoma, multiple																									
Papilloma squamous																									
Sarcoma, metastatic, uncertain primary site																									
Squamous cell carcinoma										X	X							X	X						
Squamous cell carcinoma, multiple																X									
Sebaceous gland, adenoma																X									
Sebaceous gland, adenoma, multiple										X															
Sebaceous gland, carcinoma																									
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone									+																
Sternum, hemangiosarcoma																									
Skeletal muscle								+																	
Diaphragm, mesothelioma malignant, metastatic, testes								X																	
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant									X																
Peripheral nerve		+																							
Schwannoma malignant		X																							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Hepatocellular carcinoma, metastatic, liver																									
Mesothelioma malignant, metastatic, testes								X																	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	6 2 2	6 2 5	6 3 4	6 3 8	6 3 8	6 4 9	6 5 1	6 5 8	6 6 1	6 6 3	6 6 3	6 6 3	6 6 3	6 6 7	6 7 3	6 9 3	6 9 4	6 9 4	6 9 4	7 0 4	7 1 1	7 1 1	7 2 3	7 3 4	7 3 2	
Carcass ID Number	0 9 0 3	0 8 7 4	0 8 8 3	0 8 2 3	0 8 9 3	0 8 2 2	0 8 3 3	0 8 7 3	0 8 5 1	0 8 1 2	0 8 4 3	0 8 8 2	0 8 9 2	0 8 7 2	0 8 2 1	0 8 9 1	0 8 3 2	0 8 8 1	0 9 0 2	0 8 4 2	0 8 1 1	0 9 0 1	0 8 4 1	0 8 7 1	0 8 3 1	**Total Tissues/ Tumors**
Integumentary System																										
Mammary gland	M	+	M	+	+	+	+	M	+	+	+	+	+	+	M	+	M	M	+	+	+	+	M	+	+	37
Fibroadenoma																						X				1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma		X	X			X	X		X			X												X		11
Basal cell adenoma, multiple					X					X	X		X	X	X	X	X	X			X	X	X		X	19
Basal cell carcinoma									X						X											5
Basal cell carcinoma, multiple																	X									1
Keratoacanthoma								X											X				X			5
Keratoacanthoma, multiple		X																							X	2
Papilloma squamous																				X			X			2
Sarcoma, metastatic, uncertain primary site								X																		1
Squamous cell carcinoma				X														X								6
Squamous cell carcinoma, multiple																										1
Sebaceous gland, adenoma																					X		X			3
Sebaceous gland, adenoma, multiple										X																2
Sebaceous gland, carcinoma				X																						1
Subcutaneous tissue, fibroma													X													1
Subcutaneous tissue, lipoma												X														1
Musculoskeletal System																										
Bone																+										2
Sternum, hemangiosarcoma																X										1
Skeletal muscle																										1
Diaphragm, mesothelioma malignant, metastatic, testes																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Peripheral nerve																										1
Schwannoma malignant																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																			X				X		X	3
Hepatocellular carcinoma, metastatic, liver																			X							1
Mesothelioma malignant, metastatic, testes																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	2	3	3	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6
	9	7	8	1	5	7	7	0	1	1	2	2	3	3	3	3	3	3	3	4	5	8	8	1	1
	8	7	3	2	6	0	3	1	1	2	4	4	0	0	0	1	1	1	8	5	6	1	2	4	7
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	7	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	9	8	8	8	9	8	8
	9	8	2	0	5	6	6	6	5	1	2	3	4	8	9	5	7	0	9	5	4	0	6	6	3
	5	5	5	5	5	4	5	3	4	3	4	5	5	4	5	3	5	5	4	2	4	4	2	1	4
Respiratory System (continued)																										
Lung (continued)																										
Sarcoma, metastatic, uncertain primary site																										
Squamous cell carcinoma, metastatic, skin										X	X															
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																					X					
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Eye																										
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																										
Carcinoma												X							X				X		
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, skin										X																
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X								X										X			
Lymphoma malignant																										
Mesothelioma malignant								X								X	X								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	6 2 2	6 2 5	6 3 4	6 3 8	6 3 8	6 4 9	6 5 1	6 5 8	6 6 1	6 6 3	6 6 3	6 6 3	6 6 3	6 7 7	6 9 3	6 9 3	6 9 4	6 9 4	6 9 4	7 0 4	7 1 1	7 1 1	7 2 3	7 2 4	7 3 2	
Carcass ID Number	0 9 0 3	0 8 7 4	0 8 8 3	0 8 2 3	0 8 9 3	0 8 2 2	0 8 3 3	0 8 7 3	0 8 5 1	0 8 1 2	0 8 4 3	0 8 8 2	0 8 9 2	0 8 7 2	0 8 2 1	0 8 9 1	0 8 3 2	0 8 8 1	0 8 0 2	0 8 4 2	0 8 1 1	0 9 0 1	0 8 4 1	0 8 7 1	0 8 3 1	**Total Tissues/ Tumors**
Respiratory System (continued)																										
Lung (continued)																										
Sarcoma, metastatic, uncertain primary site								X																		1
Squamous cell carcinoma, metastatic, skin																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye														+												1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X																								1
Carcinoma						X									X				X							6
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic, skin																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X		X		X	X			X	X		X									X		X	X	12
Lymphoma malignant																						X				1
Mesothelioma malignant																										3

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

	0 ppm	70 ppm	150 ppm	300 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rates[a]	16/50 (32%)	11/35 (31%)	24/63 (38%)	19/49 (39%)
Effective rates[b]	16/45 (36%)	11/32 (34%)	24/59 (41%)	19/39 (49%)
Terminal rates[c]	10/24 (42%)	8/15 (53%)	12/26 (46%)	0/1 (0%)
First incidence (days)	607	524	565	524
Life table tests[d]	P<0.001	P=0.503	P=0.216	P<0.001
Logistic regression tests[d]	P=0.008	P=0.573	P=0.312	P=0.017
Cochran-Armitage test[d]	P=0.104			
Fisher exact test[d]		P=0.555N	P=0.372	P=0.159
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rates	2/50 (4%)	0/35 (0%)	3/63 (5%)	2/49 (4%)
Effective rates	2/48 (4%)	0/34 (0%)	3/61 (5%)	2/46 (4%)
Terminal rates	0/24 (0%)	0/15 (0%)	2/26 (8%)	0/1 (0%)
First incidence (days)	607	-[e]	691	412
Life table tests	P=0.168	P=0.339N	P=0.568	P=0.511
Logistic regression tests	P=0.478	P=0.319N	P=0.607	P=0.639N
Cochran-Armitage test	P=0.449			
Fisher exact test		P=0.340N	P=0.613	P=0.675
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rates	17/50 (34%)	11/35 (31%)	27/63 (43%)	21/49 (43%)
Effective rates	17/48 (35%)	11/34 (32%)	27/61 (44%)	21/46 (46%)
Terminal rates	10/24 (42%)	8/15 (53%)	14/26 (54%)	0/1 (0%)
First incidence (days)	607	524	565	412
Life table tests	P<0.001	P=0.572	P=0.142	P<0.001
Logistic regression tests	P=0.004	P=0.542N	P=0.212	P=0.020
Cochran-Armitage test	P=0.123			
Fisher exact test		P=0.481N	P=0.231	P=0.212
Liver: Neoplastic Nodule				
Overall rates	2/50 (4%)	1/35 (3%)	10/65 (15%)	15/50 (30%)
Effective rates	2/44 (5%)	1/29 (3%)	10/60 (17%)	15/35 (43%)
Terminal rates	1/24 (4%)	1/15 (7%)	7/26 (27%)	1/1 (100%)
First incidence (days)	607	732 (T)	641	531
Life table tests	P<0.001	P=0.651N	P=0.030	P<0.001
Logistic regression tests	P<0.001	P=0.631N	P=0.042	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.654N	P=0.051	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	6/65 (9%)	7/50 (14%)
Effective rates	0/44 (0%)	1/31 (3%)	6/61 (10%)	7/38 (18%)
Terminal rates	0/24 (0%)	0/15 (0%)	5/26 (19%)	0/1 (0%)
First incidence (days)	-	694	687	530
Life table tests	P<0.001	P=0.422	P=0.023	P<0.001
Logistic regression tests	P<0.001	P=0.423	P=0.023	P=0.008
Cochran-Armitage test	P=0.001			
Fisher exact test		P=0.413	P=0.035	P=0.003

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Liver: Neoplastic Nodule or Hepatocellular Carcinoma				
Overall rates	2/50 (4%)	2/35 (6%)	15/65 (23%)	20/50 (40%)
Effective rates	2/44 (5%)	2/31 (6%)	15/61 (25%)	20/38 (53%)
Terminal rates	1/24 (4%)	1/15 (7%)	11/26 (42%)	1/1 (100%)
First incidence (days)	607	694	641	530
Life table tests	P<0.001	P=0.532	P=0.002	P<0.001
Logistic regression tests	P<0.001	P=0.553	P=0.003	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.551	P=0.005	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rates	0/50 (0%)	2/35 (6%)	1/65 (2%)	3/50 (6%)
Effective rates	0/35 (0%)	2/23 (9%)	1/48 (2%)	3/18 (17%)
Terminal rates	0/24 (0%)	1/15 (7%)	0/26 (0%)	1/1 (100%)
First incidence (days)	–	701	654	694
Life table tests	P=0.003	P=0.140	P=0.563	P<0.001
Logistic regression tests	P=0.022	P=0.148	P=0.550	P=0.002
Cochran-Armitage test	P=0.034			
Fisher exact test		P=0.153	P=0.578	P=0.035
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rates	2/50 (4%)	2/35 (6%)	2/65 (3%)	3/50 (6%)
Effective rates	2/35 (6%)	2/23 (9%)	2/48 (4%)	3/18 (17%)
Terminal rates	2/24 (8%)	1/15 (7%)	1/26 (4%)	1/1 (100%)
First incidence (days)	732 (T)	701	654	694
Life table tests	P=0.020	P=0.512	P=0.644N	P<0.001
Logistic regression tests	P=0.102	P=0.521	P=0.618N	P=0.017
Cochran-Armitage test	P=0.204			
Fisher exact test		P=0.522	P=0.565N	P=0.209
Mammary Gland: Fibroadenoma				
Overall rates	2/50 (4%)	0/35 (0%)	3/65 (5%)	1/50 (2%)
Effective rates	2/28 (7%)	0/16 (0%)	3/29 (10%)	1/5 (20%)
Terminal rates	2/24 (8%)	0/15 (0%)	3/26 (12%)	0/1 (0%)
First incidence (days)	732 (T)	–	732 (T)	711
Life table tests	P=0.142	P=0.346N	P=0.537	P=0.275
Logistic regression tests	P=0.216	P=0.346N	P=0.537	P=0.495
Cochran-Armitage test	P=0.243			
Fisher exact test		P=0.400N	P=0.517	P=0.400
Mammary Gland: Adenoma or Fibroadenoma				
Overall rates	2/50 (4%)	1/35 (3%)	3/65 (5%)	1/50 (2%)
Effective rates	2/28 (7%)	1/16 (6%)	3/29 (10%)	1/5 (20%)
Terminal rates	2/24 (8%)	1/15 (7%)	3/26 (12%)	0/1 (0%)
First incidence (days)	732 (T)	732 (T)	732 (T)	711
Life table tests	P=0.170	P=0.664N	P=0.537	P=0.275
Logistic regression tests	P=0.249	P=0.664N	P=0.537	P=0.495
Cochran-Armitage test	P=0.288			
Fisher exact test		P=0.704N	P=0.517	P=0.400

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rates	12/50 (24%)	7/35 (20%)	14/65 (22%)	4/47 (9%)
Effective rates	12/48 (25%)	7/34 (21%)	14/64 (22%)	4/45 (9%)
Terminal rates	4/24 (17%)	3/15 (20%)	7/26 (27%)	0/1 (0%)
First incidence (days)	590	530	567	383
Life table tests	P=0.426	P=0.522N	P=0.561	P=0.569
Logistic regression tests	P=0.073N	P=0.449N	P=0.463N	P=0.050N
Cochran-Armitage test	P=0.036N			
Fisher exact test		P=0.424N	P=0.434N	P=0.036N
Preputial Gland: Adenoma				
Overall rates	2/44 (5%)	1/34 (3%)	2/60 (3%)	1/48 (2%)
Effective rates	2/41 (5%)	1/32 (3%)	2/57 (4%)	1/44 (2%)
Terminal rates	2/18 (11%)	1/14 (7%)	0/22 (0%)	0/0 (0%)
First incidence (days)	732 (T)	732 (T)	565	456
Life table tests	P=0.496	P=0.589N	P=0.620N	P=0.496
Logistic regression tests	P=0.391N	P=0.589N	P=0.573N	P=0.516N
Cochran-Armitage test	P=0.378N			
Fisher exact test		P=0.593N	P=0.559N	P=0.473N
Preputial Gland: Carcinoma				
Overall rates	5/44 (11%)	3/34 (9%)	3/60 (5%)	4/48 (8%)
Effective rates	5/38 (13%)	3/30 (10%)	3/56 (5%)	4/36 (11%)
Terminal rates	3/18 (17%)	1/14 (7%)	0/22 (0%)	0/0 (0%)
First incidence (days)	680	530	530	530
Life table tests	P=0.318	P=0.545N	P=0.252N	P=0.093
Logistic regression tests	P=0.323N	P=0.502N	P=0.199N	P=0.586N
Cochran-Armitage test	P=0.437N			
Fisher exact test		P=0.496N	P=0.170N	P=0.535N
Preputial Gland: Adenoma or Carcinoma				
Overall rates	7/44 (16%)	4/34 (12%)	5/60 (8%)	5/48 (10%)
Effective rates	7/41 (17%)	4/32 (13%)	5/57 (9%)	5/44 (11%)
Terminal rates	5/18 (28%)	2/14 (14%)	0/22 (0%)	0/0 (0%)
First incidence (days)	680	530	530	456
Life table tests	P=0.274	P=0.458N	P=0.252N	P=0.053
Logistic regression tests	P=0.237N	P=0.423N	P=0.182N	P=0.449N
Cochran-Armitage test	P=0.269N			
Fisher exact test		P=0.420N	P=0.177N	P=0.329N
Skin: Basal Cell Adenoma				
Overall rates	1/50 (2%)	4/35 (11%)	26/65 (40%)	30/50 (60%)
Effective rates	1/46 (2%)	4/32 (13%)	26/62 (42%)	30/44 (68%)
Terminal rates	1/24 (4%)	3/15 (20%)	19/26 (73%)	1/1 (100%)
First incidence (days)	732 (T)	651	641	473
Life table tests	P<0.001	P=0.071	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.073	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.088	P<0.001	P<0.001

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Skin: Basal Cell Carcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	5/65 (8%)	6/50 (12%)
Effective rates	0/46 (0%)	1/32 (3%)	5/62 (8%)	6/44 (14%)
Terminal rates	0/24 (0%)	0/15 (0%)	4/26 (15%)	0/1 (0%)
First incidence (days)	–	724	673	473
Life table tests	P<0.001	P=0.411	P=0.043	P=0.002
Logistic regression tests	P=0.003	P=0.408	P=0.046	P=0.020
Cochran-Armitage test	P=0.005			
Fisher exact test		P=0.410	P=0.058	P=0.011
Skin: Basal Cell Adenoma or Carcinoma				
Overall rates	1/50 (2%)	5/35 (14%)	28/65 (43%)	32/50 (64%)
Effective rates	1/46 (2%)	5/32 (16%)	28/62 (45%)	32/44 (73%)
Terminal rates	1/24 (4%)	3/15 (20%)	20/26 (77%)	1/1 (100%)
First incidence (days)	732 (T)	651	641	473
Life table tests	P<0.001	P=0.032	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.030	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.040	P<0.001	P<0.001
Skin: Keratoacanthoma				
Overall rates	1/50 (2%)	1/35 (3%)	4/65 (6%)	7/50 (14%)
Effective rates	1/42 (2%)	1/28 (4%)	4/58 (7%)	7/28 (25%)
Terminal rates	1/24 (4%)	1/15 (7%)	4/26 (15%)	1/1 (100%)
First incidence (days)	732 (T)	732 (T)	732 (T)	582
Life table tests	P<0.001	P=0.654	P=0.200	P<0.001
Logistic regression tests	P<0.001	P=0.654	P=0.200	P=0.005
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.643	P=0.298	P=0.006
Skin: Squamous Papilloma				
Overall rates	1/50 (2%)	0/35 (0%)	3/65 (5%)	2/50 (4%)
Effective rates	1/35 (3%)	0/23 (0%)	3/48 (6%)	2/18 (11%)
Terminal rates	1/24 (4%)	0/15 (0%)	1/26 (4%)	0/1 (0%)
First incidence (days)	732 (T)	–	654	704
Life table tests	P=0.020	P=0.594N	P=0.368	P=0.012
Logistic regression tests	P=0.093	P=0.594N	P=0.401	P=0.078
Cochran-Armitage test	P=0.109			
Fisher exact test		P=0.603N	P=0.435	P=0.263
Skin: Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	2/35 (6%)	8/65 (12%)	7/50 (14%)
Effective rates	0/45 (0%)	2/32 (6%)	8/61 (13%)	7/41 (17%)
Terminal rates	0/24 (0%)	2/15 (13%)	5/26 (19%)	0/1 (0%)
First incidence (days)	–	732 (T)	565	512
Life table tests	P<0.001	P=0.141	P=0.009	P=0.002
Logistic regression tests	P=0.006	P=0.141	P=0.013	P=0.017
Cochran-Armitage test	P=0.004			
Fisher exact test		P=0.170	P=0.010	P=0.004

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Skin: Squamous Papilloma or Squamous Cell Carcinoma				
Overall rates	1/50 (2%)	2/35 (6%)	11/65 (17%)	9/50 (18%)
Effective rates	1/45 (2%)	2/32 (6%)	11/61 (18%)	9/41 (22%)
Terminal rates	1/24 (4%)	2/15 (13%)	6/26 (23%)	0/1 (0%)
First incidence (days)	732 (T)	732 (T)	565	512
Life table tests	P<0.001	P=0.336	P=0.007	P<0.001
Logistic regression tests	P=0.001	P=0.336	P=0.010	P=0.011
Cochran-Armitage test	P=0.002			
Fisher exact test		P=0.373	P=0.009	P=0.005
Skin: (Sebaceous Gland): Adenoma or Carcinoma				
Overall rates	1/50 (2%)	1/35 (3%)	5/65 (8%)	6/50 (12%)
Effective rates	1/45 (2%)	1/32 (3%)	5/61 (8%)	6/41 (15%)
Terminal rates	0/24 (0%)	1/15 (7%)	4/26 (15%)	0/1 (0%)
First incidence (days)	706	732 (T)	593	512
Life table tests	P<0.001	P=0.638	P=0.132	P=0.001
Logistic regression tests	P=0.007	P=0.663	P=0.166	P=0.036
Cochran-Armitage test	P=0.014			
Fisher exact test		P=0.662	P=0.189	P=0.042
Skin (Subcutaneous Tissue): Fibroma				
Overall rates	4/50 (8%)	3/35 (9%)	5/65 (8%)	1/50 (2%)
Effective rates	4/39 (10%)	3/28 (11%)	5/54 (9%)	1/23 (4%)
Terminal rates	3/24 (13%)	0/15 (0%)	2/26 (8%)	0/1 (0%)
First incidence (days)	641	631	641	663
Life table tests	P=0.583N	P=0.593	P=0.580	P=0.647
Logistic regression tests	P=0.239N	P=0.616	P=0.629N	P=0.449N
Cochran-Armitage test	P=0.283N			
Fisher exact test		P=0.627	P=0.570N	P=0.381N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rates	5/50 (10%)	3/35 (9%)	5/65 (8%)	1/50 (2%)
Effective rates	5/39 (13%)	3/28 (11%)	5/54 (9%)	1/23 (4%)
Terminal rates	3/24 (13%)	0/15 (0%)	2/26 (8%)	0/1 (0%)
First incidence (days)	641	631	641	663
Life table tests	P=0.469N	P=0.594N	P=0.553N	P=0.705
Logistic regression tests	P=0.156N	P=0.569N	P=0.473N	P=0.328N
Cochran-Armitage test	P=0.187N			
Fisher exact test		P=0.554N	P=0.413N	P=0.268N
Testes: Adenoma				
Overall rates	44/50 (88%)	35/35 (100%)	59/64 (92%)	43/50 (86%)
Effective rates	44/49 (90%)	35/35 (100%)	59/63 (94%)	43/49 (88%)
Terminal rates	23/24 (96%)	15/15 (100%)	25/26 (96%)	1/1 (100%)
First incidence (days)	454	352	499	456
Life table tests	P<0.001	P=0.129	P=0.201	P<0.001
Logistic regression tests	P=0.489	P=0.045	P=0.468	P=0.288
Cochran-Armitage test	P=0.241N			
Fisher exact test		P=0.062	P=0.344	P=0.500N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Thyroid Gland (C-cell): Adenoma				
Overall rates	5/50 (10%)	2/35 (6%)	6/65 (9%)	3/49 (6%)
Effective rates	5/44 (11%)	2/31 (6%)	6/61 (10%)	3/37 (8%)
Terminal rates	2/24 (8%)	2/15 (13%)	3/26 (12%)	0/1 (0%)
First incidence (days)	636	732 (T)	530	638
Life table tests	P=0.163	P=0.436N	P=0.594	P=0.283
Logistic regression tests	P=0.553	P=0.398N	P=0.568N	P=0.651N
Cochran-Armitage test	P=0.442N			
Fisher exact test		P=0.384N	P=0.522N	P=0.458N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rates	6/50 (12%)	3/35 (9%)	6/65 (9%)	4/49 (8%)
Effective rates	6/44 (14%)	3/31 (10%)	6/61 (10%)	4/37 (11%)
Terminal rates	2/24 (8%)	2/15 (13%)	3/26 (12%)	0/1 (0%)
First incidence (days)	636	666	530	638
Life table tests	P=0.135	P=0.510N	P=0.524N	P=0.147
Logistic regression tests	P=0.544	P=0.463N	P=0.428N	P=0.568
Cochran-Armitage test	P=0.430N			
Fisher exact test		P=0.444N	P=0.381N	P=0.485N
Thyroid Gland (Follicular Cell): Carcinoma				
Overall rates	2/50 (4%)	2/35 (6%)	2/65 (3%)	0/49 (0%)
Effective rates	2/43 (5%)	2/28 (7%)	2/60 (3%)	0/28 (0%)
Terminal rates	1/24 (4%)	1/15 (7%)	0/26 (0%)	0/1 (0%)
First incidence (days)	715	651	565	–
Life table tests	P=0.345N	P=0.521	P=0.625N	P=0.842N
Logistic regression tests	P=0.144N	P=0.542	P=0.605N	P=0.632N
Cochran-Armitage test	P=0.189N			
Fisher exact test		P=0.517	P=0.557N	P=0.363N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rates	2/50 (4%)	2/35 (6%)	3/65 (5%)	2/49 (4%)
Effective rates	2/45 (4%)	2/32 (6%)	3/61 (5%)	2/39 (5%)
Terminal rates	1/24 (4%)	1/15 (7%)	1/26 (4%)	1/1 (100%)
First incidence (days)	715	651	565	524
Life table tests	P=0.195	P=0.521	P=0.573	P=0.093
Logistic regression tests	P=0.572	P=0.542	P=0.618	P=0.536
Cochran-Armitage test	P=0.575			
Fisher exact test		P=0.554	P=0.642	P=0.636
Zymbal's Gland: Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	7/65 (11%)	6/50 (12%)
Effective rates	0/49 (0%)	0/35 (0%)	7/65 (11%)	6/49 (12%)
Terminal rates	0/24 (0%)	0/15 (0%)	2/26 (8%)	0/1 (0%)
First incidence (days)	–	–	325	524
Life table tests	P<0.001	–	P=0.022	P=0.002
Logistic regression tests	P=0.011	–	P=0.013	P=0.018
Cochran-Armitage test	P=0.004			
Fisher exact test		–	P=0.017	P=0.013

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Zymbal's Gland: Adenoma or Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	8/65 (12%)	7/50 (14%)
Effective rates	0/49 (0%)	0/35 (0%)	8/65 (12%)	7/49 (14%)
Terminal rates	0/24 (0%)	0/15 (0%)	2/26 (8%)	0/1 (0%)
First incidence (days)	–	–	325	524
Life table tests	P<0.001	–	P=0.014	P<0.001
Logistic regression tests	P=0.005	–	P=0.008	P=0.009
Cochran-Armitage test	P=0.002			
Fisher exact test		–	P=0.009	P=0.006
All Organs: Mononuclear Cell Leukemia				
Overall rates	20/50 (40%)	20/35 (57%)	37/65 (57%)	12/50 (24%)
Effective rates	20/49 (41%)	20/35 (57%)	37/64 (58%)	12/49 (24%)
Terminal rates	9/24 (38%)	8/15 (53%)	11/26 (42%)	1/1 (100%)
First incidence (days)	564	352	455	456
Life table tests	P=0.021	P=0.081	P=0.053	P=0.027
Logistic regression tests	P=0.112N	P=0.084	P=0.057	P=0.261N
Cochran-Armitage test	P=0.029N			
Fisher exact test		P=0.105	P=0.054	P=0.065N
All Organs: Mesothelioma Malignant				
Overall rates	4/50 (8%)	6/35 (17%)	4/65 (6%)	3/50 (6%)
Effective rates	4/48 (8%)	6/34 (18%)	4/63 (6%)	3/46 (7%)
Terminal rates	1/24 (4%)	2/15 (13%)	2/26 (8%)	0/1 (0%)
First incidence (days)	458	447	693	501
Life table tests	P=0.476	P=0.161	P=0.571N	P=0.510
Logistic regression tests	P=0.169N	P=0.165	P=0.499N	P=0.382N
Cochran-Armitage test	P=0.253N			
Fisher exact test		P=0.177	P=0.482N	P=0.524N
All Organs: Benign Tumors				
Overall rates	48/50 (96%)	35/35 (100%)	61/65 (94%)	45/50 (90%)
Effective rates	48/49 (98%)	35/35 (100%)	61/64 (95%)	45/49 (92%)
Terminal rates	24/24 (100%)	15/15 (100%)	26/26 (100%)	1/1 (100%)
First incidence (days)	454	352	499	383
Life table tests	P<0.001	P=0.265	P=0.309	P<0.001
Logistic regression tests	P=0.211N	P=0.477	P=0.165N	P=0.421N
Cochran-Armitage test	P=0.055N			
Fisher exact test		P=0.583	P=0.416N	P=0.181N
All Organs: Malignant Tumors				
Overall rates	33/50 (66%)	27/35 (77%)	54/65 (83%)	43/50 (86%)
Effective rates	33/50 (66%)	27/35 (77%)	54/65 (83%)	43/50 (86%)
Terminal rates	14/24 (58%)	10/15 (67%)	19/26 (73%)	1/1 (100%)
First incidence (days)	131	352	325	377
Life table tests	P<0.001	P=0.185	P=0.055	P<0.001
Logistic regression tests	P=0.022	P=0.199	P=0.027	P=0.019
Cochran-Armitage test	P=0.011			
Fisher exact test		P=0.193	P=0.029	P=0.017

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
All Organs: Benign or Malignant Tumors				
Overall rates	49/50 (98%)	35/35 (100%)	64/65 (98%)	49/50 (98%)
Effective rates	49/50 (98%)	35/35 (100%)	64/65 (98%)	49/50 (98%)
Terminal rates	24/24 (100%)	15/15 (100%)	26/26 (100%)	1/1 (100%)
First incidence (days)	131	352	325	377
Life table tests	P<0.001	P=0.311	P=0.253	P<0.001
Logistic regression tests	P=0.605N	P=0.724	P=0.765N	P=0.776N
Cochran-Armitage test	P=0.569N			
Fisher exact test		P=0.588	P=0.683	P=0.753N

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at first occurance of this tumor type in any of the groups
[c] Observed incidence at terminal kill
[d] Beneath the "0 ppm" column are the P values associated with the trend test. Beneath the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the effective incidence rates. For all tests, a negative trend or a lower incidence in a dosed group is indicated by N.
[e] Not applicable; no tumors in animal group

TABLE A4a
Historical Incidence of Skin Keratoacanthomas in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Overall Historical Incidence: Feed and Water	
Total	34/681 (5.0%)
Standard deviation	3.0%
Range	2%-11%

[a] Data as of 17 September 1990

TABLE A4b
Historical Incidence of Sebaceous Gland Adenomas in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Overall Historical Incidence: Feed and Water	
Total	3/681 (0.4%)
Standard deviation	0.9%
Range	0%-2%

[a] Data as of 17 September 1990; includes data for sebaceous gland carcinoma, basal cell carcinoma or adenoma

TABLE A4c
Historical Incidence of Skin Basal Cell Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	3/681 (0.4%)	3/681 (0.4%)	6/681 (0.9%)
Standard deviation	0.8%	1.7%	1.3%
Range	0%-2%	0%-6%	0%-6%

[a] Data as of 17 September 1990

TABLE A4d
Historical Incidence of Skin Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Squamous Cell Carcinoma
Overall Historical Incidence: Feed and Water			
Total	13/681 (1.9%)	5/681 (0.7%)	17/681 (2.5%)
Standard deviation	1.4%	1.3%	1.5%
Range	0%-4%	0%-4%	0%-4%

[a] Data as of 17 September 1990

TABLE A4e
Historical Incidence of Zymbal's Gland Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	1/681 (0.1%)	10/681 (1.5%)	11/681 (1.6%)
Standard deviation	0.6%	1.5%	1.7%
Range	0%-2%	0%-4%	0%-4%

[a] Data as of 17 September 1990

TABLE A4f
Historical Incidence of Liver Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	17/680 (2.5%)	7/680 (1.0%)	22/680 (3.2%)
Standard deviation	2.9%	1.9%	3.5%
Range	0%-8%	0%-6%	0%-10%

[a] Data as of 17 September 1990

TABLE A4g
Historical Incidence of Lung Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Alveolar/bronchiolar Adenoma	Alveolar/bronchiolar Carcinoma	Alveolar/bronchiolar Adenoma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	15/680 (2.2%)	5/680 (0.7%)	22/680[b] (3.2%)
Standard deviation	2.3%	1.3%	2.6%
Range	0%-6%	0%-2%	0%-10%

[a] Data as of 17 September 1990
[b] Includes one adenosquamous carcinoma

TABLE A4h
Historical Incidence of Oral Cavity Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Papilloma or Squamous Cell Papilloma	Squamous Cell Carcinoma	Papilloma, Squamous Cell Papilloma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	4/681 (0.6%)	0/681 (0.0%)	4/681 (0.6%)
Standard deviation	1.5%		1.5%
Range	0%-4%		0%-4%

[a] Data as of 17 September 1990; includes tongue, pharynx (palate), tooth (gingiva), and lip.

TABLE A4i
Historical Incidence of Adrenal Medulla Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Benign Pheochromocytoma	Malignant Pheochromocytoma	Benign or Malignant Pheochromocytoma
Overall Historical Incidence: Feed and Water			
Total	250/672 (37.2%)	27/672 (4.0%)	267/672[b] (39.7%)
Standard deviation	7.5%	5.6%	7.8%
Range	22%-47%	0%-20%	22%-49%

[a] Data as of 17 September 1990
[b] Numerator includes two complex pheochromocytomas, one occurring in a feed study and one occurring in a drinking water study.

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

	0 ppm	70 ppm	150 ppm	300 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
Interim evaluations	20	10	10	20
Early deaths				
Natural death	13	11	16	16
Moribund	13	9	23	33
Survivors				
Terminal sacrifice	23	15	26	1
Died last week of studies	1			
Animals examined microscopically	50	35	65	50
Alimentary System				
Intestine large, cecum	(50)	(35)	(64)	(49)
Edema				2 (4%)
Hemorrhage		1 (3%)		
Intestine large, colon	(50)	(35)	(64)	(50)
Diverticulum	1 (2%)			
Erosion			1 (2%)	
Parasite metazoan	3 (6%)	1 (3%)	6 (9%)	3 (6%)
Intestine large, rectum	(50)	(34)	(64)	(50)
Inflammation, chronic active		1 (3%)		
Inflammation, suppurative		1 (3%)		
Parasite metazoan	3 (6%)	3 (9%)	4 (6%)	3 (6%)
Ulcer	1 (2%)			
Intestine small, ileum	(50)	(35)	(64)	(49)
Inflammation, granulomatous	1 (2%)			
Ulcer		1 (3%)		
Liver	(50)	(35)	(65)	(50)
Angiectasis	3 (6%)	2 (6%)	2 (3%)	
Basophilic focus	16 (32%)	15 (43%)	23 (35%)	25 (50%)
Clear cell focus	2 (4%)		2 (3%)	2 (4%)
Cyst				1 (2%)
Cytoplasmic alteration, focal		1 (3%)		
Degeneration, cystic	6 (12%)	13 (37%)	33 (51%)	31 (62%)
Eosinophilic focus	6 (12%)	5 (14%)	19 (29%)	26 (52%)
Fatty change, diffuse	1 (2%)			
Fatty change, focal	1 (2%)		2 (3%)	
Fatty change, multifocal			2 (3%)	5 (10%)
Hematocyst			1 (2%)	
Hematopoietic cell proliferation	1 (2%)	2 (6%)	6 (9%)	14 (28%)
Hepatodiaphragmatic nodule	3 (6%)	1 (3%)	8 (12%)	4 (8%)
Hepatodiaphragmatic nodule, multiple	1 (2%)			
Infarct	1 (2%)	1 (3%)	4 (6%)	5 (10%)
Inflammation, granulomatous, multifocal	1 (2%)		1 (2%)	1 (2%)
Mixed cell focus	7 (14%)	9 (26%)	24 (37%)	14 (28%)
Necrosis, coagulative, focal			1 (2%)	
Necrosis, coagulative, multifocal			1 (2%)	3 (6%)
Regeneration	2 (4%)	1 (3%)	3 (5%)	1 (2%)
Bile duct, cyst	1 (2%)		1 (2%)	
Bile duct, hyperplasia	33 (66%)	23 (66%)	31 (48%)	10 (20%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Alimentary System (continued)				
Liver (continued)				
Centrilobular, fatty change		3 (9%)	2 (3%)	2 (4%)
Centrilobular, hemorrhage	1 (2%)			
Centrilobular, necrosis, coagulative	3 (6%)	3 (9%)	8 (12%)	18 (36%)
Centrilobular, necrosis, coagulative, focal				1 (2%)
Periportal, fatty change	1 (2%)			
Portal vein, thrombus			1 (2%)	
Serosa, fibrosis, focal	2 (4%)			
Serosa, pigmentation, focal	2 (4%)			
Mesentery	(11)	(6)	(1)	(6)
Hemorrhage	1 (9%)			
Artery, inflammation, chronic active	1 (9%)			
Fat, necrosis, focal	8 (73%)	3 (50%)	1 (100%)	5 (83%)
Pancreas	(50)	(35)	(64)	(49)
Metaplasia				1 (2%)
Acinus, atrophy	13 (26%)	7 (20%)	18 (28%)	7 (14%)
Acinus, hyperplasia, focal	1 (2%)	1 (3%)		2 (4%)
Artery, inflammation, chronic active	1 (2%)			
Salivary glands	(49)	(35)	(64)	(50)
Edema				1 (2%)
Stomach, forestomach	(50)	(35)	(65)	(50)
Erosion			1 (2%)	
Hyperplasia	2 (4%)			
Inflammation, acute			2 (3%)	
Inflammation, chronic active			1 (2%)	
Ulcer	4 (8%)	1 (3%)	2 (3%)	2 (4%)
Stomach, glandular	(50)	(35)	(65)	(50)
Degeneration, cystic				1 (2%)
Erosion	1 (2%)		3 (5%)	6 (12%)
Inflammation, chronic	1 (2%)	1 (3%)		
Inflammation, suppurative, focal			1 (2%)	
Ulcer			2 (3%)	
Artery, inflammation, chronic active	1 (2%)			
Tooth	(1)			
Dysplasia	1 (100%)			
Cardiovascular System				
Heart	(50)	(35)	(65)	(50)
Cardiomyopathy	24 (48%)	17 (49%)	36 (55%)	24 (48%)
Inflammation, suppurative				1 (2%)
Atrium, dilatation	1 (2%)	2 (6%)	1 (2%)	1 (2%)
Atrium, thrombus	5 (10%)	4 (11%)	18 (28%)	18 (36%)
Ventricle right, thrombus				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Endocrine System				
Adrenal gland	(50)	(35)	(63)	(50)
Bilateral, hyperplasia, focal				2 (4%)
Adrenal gland, cortex	(50)	(35)	(63)	(50)
Angiectasis	1 (2%)		2 (3%)	
Atrophy			1 (2%)	
Necrosis, diffuse			1 (2%)	
Vacuolization cytoplasmic, diffuse	1 (2%)			
Vacuolization cytoplasmic, focal	2 (4%)	1 (3%)	8 (13%)	3 (6%)
Vacuolization cytoplasmic, multifocal	1 (2%)	2 (6%)		2 (4%)
Adrenal gland, medulla	(50)	(35)	(63)	(49)
Hyperplasia, focal	11 (22%)	5 (14%)	20 (32%)	11 (22%)
Hyperplasia, multifocal	1 (2%)	1 (3%)	6 (10%)	2 (4%)
Necrosis, diffuse			1 (2%)	
Bilateral, hyperplasia, focal		1 (3%)	1 (2%)	
Bilateral, hyperplasia, multifocal			1 (2%)	
Pituitary gland	(50)	(35)	(65)	(47)
Angiectasis	2 (4%)	2 (6%)	3 (5%)	1 (2%)
Cyst	5 (10%)	3 (9%)	3 (5%)	1 (2%)
Ectopic tissue				1 (2%)
Hemorrhage			1 (2%)	
Pigmentation, hemosiderin	1 (2%)			
Pars distalis, hyperplasia, focal	1 (2%)	1 (3%)	2 (3%)	3 (6%)
Thyroid gland	(50)	(35)	(65)	(49)
Ultimobranchial cyst		1 (3%)	2 (3%)	
C-cell, hyperplasia, focal	1 (2%)	1 (3%)	4 (6%)	
Follicle, cyst			1 (2%)	1 (2%)
Follicular cell, hyperplasia, focal		1 (3%)	2 (3%)	
General Body System				
Tissue NOS	(1)		(3)	(3)
Inflammation, chronic	1 (100%)		1 (33%)	
Thrombus, chronic	1 (100%)			
Genital System				
Epididymis	(50)	(35)	(64)	(50)
Granuloma sperm	1 (2%)			
Inflammation, chronic active			1 (2%)	
Preputial gland	(44)	(34)	(60)	(48)
Hyperplasia, glandular, focal			1 (2%)	1 (2%)
Hyperplasia, squamous, focal		1 (3%)		
Inflammation, chronic active	1 (2%)		3 (5%)	5 (10%)
Inflammation, suppurative	1 (2%)	1 (3%)	3 (5%)	1 (2%)
Duct, ectasia	3 (7%)		2 (3%)	4 (8%)
Prostate	(50)	(34)	(64)	(50)
Atrophy	2 (4%)	4 (12%)	3 (5%)	
Hyperplasia, focal	2 (4%)	6 (18%)	10 (16%)	2 (4%)
Hyperplasia, multifocal	3 (6%)	1 (3%)	3 (5%)	
Infiltration cellular, lymphocytic			1 (2%)	
Inflammation, chronic active	1 (2%)	1 (3%)	3 (5%)	8 (16%)
Inflammation, suppurative	2 (4%)	3 (9%)		1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Genital System (continued)				
Seminal vesicle	(49)	(34)	(65)	(48)
Atrophy	30 (61%)	24 (71%)	51 (78%)	18 (38%)
Inflammation, chronic active	1 (2%)			1 (2%)
Inflammation, suppurative		1 (3%)		
Testes	(50)	(35)	(64)	(50)
Giant cell	1 (2%)		1 (2%)	
Granuloma sperm			1 (2%)	
Hypospermia	5 (10%)	8 (23%)	6 (9%)	3 (6%)
Mineralization			1 (2%)	
Artery, inflammation, chronic active	1 (2%)			
Bilateral, hypospermia	2 (4%)			
Interstitial cell, hyperplasia	6 (12%)	3 (9%)	3 (5%)	8 (16%)
Hematopoietic System				
Bone marrow	(50)	(35)	(64)	(50)
Atrophy	4 (8%)	1 (3%)		1 (2%)
Hemorrhage	2 (4%)			
Myelofibrosis		1 (3%)	1 (2%)	1 (2%)
Lymph node	(49)	(35)	(65)	(50)
Mediastinal, hemorrhage	7 (14%)	8 (23%)	5 (8%)	11 (22%)
Mediastinal, pigmentation, hemosiderin	1 (2%)	1 (3%)		1 (2%)
Mediastinal, sinus, ectasia			1 (2%)	
Pancreatic, hemorrhage	1 (2%)			1 (2%)
Lymph node, mandibular	(49)	(35)	(65)	(49)
Fibrosis				1 (2%)
Hemorrhage	4 (8%)	1 (3%)	2 (3%)	2 (4%)
Hyperplasia, lymphoid				1 (2%)
Hyperplasia, plasma cell	8 (16%)	8 (23%)	2 (3%)	9 (18%)
Sinus, ectasia	5 (10%)	7 (20%)	13 (20%)	6 (12%)
Lymph node, mesenteric	(49)	(35)	(64)	(50)
Fibrosis				1 (2%)
Hemorrhage	1 (2%)			2 (4%)
Hyperplasia, lymphoid				1 (2%)
Hyperplasia, RE cell				1 (2%)
Sinus, ectasia	1 (2%)	1 (3%)		
Spleen	(50)	(35)	(64)	(50)
Ectopic tissue			1 (2%)	
Edema		1 (3%)		
Fibrosis	1 (2%)	3 (9%)	2 (3%)	3 (6%)
Hematopoietic cell proliferation	3 (6%)	4 (11%)	3 (5%)	21 (42%)
Hemorrhage				1 (2%)
Necrosis	1 (2%)	1 (3%)	1 (2%)	1 (2%)
Pigmentation, hemosiderin	1 (2%)			
Red pulp, atrophy			2 (3%)	
Thymus	(41)	(22)	(47)	(40)
Congestion			1 (2%)	
Hemorrhage	3 (7%)	2 (9%)		2 (5%)
Epithelial cell, hyperplasia	20 (49%)	8 (36%)	19 (40%)	14 (35%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Integumentary System				
Mammary gland	(46)	(31)	(61)	(37)
Galactocele		1 (3%)		1 (3%)
Hyperplasia, nodular	1 (2%)		1 (2%)	
Skin	(50)	(35)	(65)	(50)
Cyst epithelial inclusion	1 (2%)	1 (3%)		1 (2%)
Hyperkeratosis, focal			1 (2%)	1 (2%)
Ulcer	1 (2%)	1 (3%)		
Epidermis, hyperplasia, basal cell, focal		1 (3%)		
Hair follicle, hyperplasia, basal cell, focal				1 (2%)
Sebaceous gland, hyperplasia, focal			3 (5%)	1 (2%)
Subcutaneous tissue, edema			1 (2%)	2 (4%)
Subcutaneous tissue, inflammation, acute			2 (3%)	
Musculoskeletal System				
Bone				(2)
Sternum, osteopetrosis				1 (50%)
Nervous System				
Brain	(50)	(35)	(64)	(50)
Hemorrhage	5 (10%)	2 (6%)	7 (11%)	2 (4%)
Inflammation, suppurative				1 (2%)
Brain stem, compression	3 (6%)	2 (6%)	3 (5%)	1 (2%)
Respiratory System				
Lung	(50)	(35)	(65)	(50)
Congestion	3 (6%)		1 (2%)	2 (4%)
Granuloma, multifocal		1 (3%)		
Hemorrhage	1 (2%)		1 (2%)	3 (6%)
Infiltration cellular, histiocytic	9 (18%)	11 (31%)	18 (28%)	9 (18%)
Pigmentation, focal				1 (2%)
Alveolar epithelium, hyperplasia, focal	2 (4%)	4 (11%)	5 (8%)	6 (12%)
Alveolar epithelium, hyperplasia, multifocal			4 (6%)	2 (4%)
Bronchiole, inflammation, acute	1 (2%)			2 (4%)
Capillary, thrombus			1 (2%)	2 (4%)
Interstitium, inflammation	4 (8%)	1 (3%)	4 (6%)	7 (14%)
Nose	(50)	(35)	(65)	(50)
Foreign body				1 (2%)
Fungus	2 (4%)	7 (20%)	8 (12%)	5 (10%)
Hemorrhage	1 (2%)		1 (2%)	
Hyperplasia, focal			1 (2%)	1 (2%)
Hyperplasia, multifocal			1 (2%)	
Inflammation, suppurative	10 (20%)	11 (31%)	15 (23%)	14 (28%)
Ulcer				1 (2%)
Trachea	(50)	(35)	(65)	(50)
Inflammation, suppurative				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	70 ppm	150 ppm	300 ppm
Special Senses System				
Eye		(1)	(2)	(1)
Cataract			2 (100%)	1 (100%)
Necrosis		1 (100%)		
Retina, degeneration			2 (100%)	
Zymbal's gland	(50)	(35)	(64)	(50)
Ectasia				2 (4%)
Hyperplasia, squamous, focal				1 (2%)
Inflammation, suppurative			2 (3%)	
Urinary System				
Kidney	(50)	(35)	(65)	(50)
Cyst		1 (3%)	1 (2%)	1 (2%)
Hydronephrosis	1 (2%)			
Infarct			1 (2%)	1 (2%)
Inflammation, suppurative				1 (2%)
Nephropathy	47 (94%)	34 (97%)	65 (100%)	46 (92%)
Pigmentation	3 (6%)	2 (6%)	2 (3%)	
Interstitial tissue, fibrosis, focal		1 (3%)		
Urinary bladder	(50)	(34)	(64)	(50)
Ectasia	1 (2%)		1 (2%)	1 (2%)
Hemorrhage	1 (2%)		2 (3%)	

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF C.I. ACID RED 114

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

	0 ppm	150 ppm	300 ppm	600 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
Interim evaluations	20	10	10	20
Early deaths				
Natural death	4	6	17	13
Moribund	10	16	41	37
Accident death	0	0	1	0
Survivors				
Terminal sacrifice	36	13	6	0
Animals examined microscopically	50	35	65	50
Alimentary System				
Esophagus	(50)	(35)	(64)	(50)
Intestine large, cecum	(50)	(35)	(63)	(49)
Intestine large, colon	(50)	(35)	(63)	(48)
Adenocarcinoma, cystic, mucinous				1 (2%)
Polyp adenomatous		1 (3%)		1 (2%)
Intestine large, rectum	(50)	(35)	(64)	(49)
Polyp adenomatous				1 (2%)
Intestine small, duodenum	(50)	(35)	(63)	(50)
Adenocarcinoma, cystic, mucinous				1 (2%)
Polyp adenomatous				1 (2%)
Intestine small, ileum	(50)	(35)	(63)	(50)
Intestine small, jejunum	(50)	(35)	(63)	(50)
Polyp adenomatous			1 (2%)	
Liver	(50)	(35)	(64)	(50)
Hepatocellular carcinoma			6 (9%)	3 (6%)
Histiocytic sarcoma			1 (2%)	1 (2%)
Neoplastic nodule			11 (17%)	6 (12%)
Neoplastic nodule, multiple			4 (6%)	
Sarcoma, metastatic, uncertain primary site			1 (2%)	
Mesentery	(4)	(1)	(4)	(11)
Schwannoma malignant				1 (9%)
Pancreas	(50)	(35)	(62)	(50)
Pharynx		(1)	(5)	(4)
Papilloma squamous		1 (100%)	4 (80%)	1 (25%)
Squamous cell carcinoma			1 (20%)	2 (50%)
Salivary glands	(48)	(35)	(64)	(50)
Schwannoma malignant	1 (2%)			
Stomach, forestomach	(50)	(35)	(64)	(50)
Sarcoma			1 (2%)	
Stomach, glandular	(50)	(35)	(64)	(50)
Tongue		(2)	(4)	(4)
Papilloma squamous		2 (100%)	2 (50%)	3 (75%)
Squamous cell carcinoma			2 (50%)	
Tooth		(1)	(1)	(2)
Odontoma				2 (100%)
Peridontal tissue, histiocytic sarcoma			1 (100%)	
Cardiovascular System				
Heart	(50)	(35)	(64)	(49)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Endocrine System				
Adrenal gland	(50)	(35)	(64)	(50)
Pheochromocytoma benign			1 (2%)	
Adrenal gland, cortex	(50)	(35)	(64)	(50)
Adenoma			1 (2%)	
Adrenal gland, medulla	(50)	(35)	(64)	(50)
Pheochromocytoma malignant			1 (2%)	
Pheochromocytoma benign	1 (2%)	3 (9%)	3 (5%)	1 (2%)
Islets, pancreatic	(50)	(35)	(63)	(49)
Adenoma	1 (2%)			
Carcinoma			1 (2%)	
Pituitary gland	(50)	(35)	(64)	(49)
Pars distalis, adenoma	25 (50%)	17 (49%)	17 (27%)	5 (10%)
Thyroid gland	(50)	(35)	(64)	(50)
C-cell, adenoma	6 (12%)	2 (6%)	2 (3%)	3 (6%)
C-cell, carcinoma	6 (12%)		2 (3%)	
Follicular cell, adenoma		1 (3%)	3 (5%)	
Follicular cell, carcinoma		2 (6%)		1 (2%)
General Body System				
Tissue NOS			(1)	(2)
Genital System				
Clitoral gland	(48)	(32)	(62)	(50)
Adenoma	7 (15%)	9 (28%)	10 (16%)	10 (20%)
Carcinoma	4 (8%)	9 (28%)	17 (27%)	13 (26%)
Bilateral, adenoma		1 (3%)		
Bilateral, carcinoma			2 (3%)	2 (4%)
Ovary	(50)	(35)	(64)	(49)
Granulosa cell tumor NOS			1 (2%)	
Periovarian tissue, sarcoma stromal, metastatic, uterus			1 (2%)	
Uterus	(50)	(35)	(64)	(49)
Adenoma			1 (2%)	
Polyp stromal	4 (8%)	6 (17%)	7 (11%)	2 (4%)
Polyp stromal, multiple		2 (6%)	1 (2%)	
Sarcoma stromal	1 (2%)	2 (6%)	2 (3%)	
Hematopoietic System				
Bone marrow	(49)	(35)	(63)	(50)
Histiocytic sarcoma				1 (2%)
Lymph node	(50)	(35)	(64)	(49)
Schwannoma malignant, metastatic, peripheral nerve				1 (2%)
Mediastinal, histiocytic sarcoma			1 (2%)	
Lymph node, mandibular	(46)	(35)	(64)	(48)
Histiocytic sarcoma			1 (2%)	
Lymph node, mesenteric	(50)	(35)	(64)	(48)
Spleen	(50)	(35)	(63)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Thymus	(40)	(30)	(59)	(42)
Thymoma benign	1 (3%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Integumentary System				
Mammary gland	(49)	(35)	(58)	(45)
Adenoacanthoma		1 (3%)		
Adenocarcinoma		3 (9%)	6 (10%)	2 (4%)
Adenocarcinoma, multiple				1 (2%)
Adenoma	1 (2%)	1 (3%)	4 (7%)	
Fibroadenoma	16 (33%)	10 (29%)	8 (14%)	
Fibroadenoma, multiple	3 (6%)	3 (9%)	4 (7%)	1 (2%)
Sarcoma			1 (2%)	
Skin	(50)	(35)	(65)	(49)
Basal cell adenoma		2 (6%)	5 (8%)	3 (6%)
Basal cell adenoma, multiple		1 (3%)		
Basal cell carcinoma		1 (3%)	2 (3%)	2 (4%)
Keratoacanthoma				1 (2%)
Papilloma squamous			1 (2%)	1 (2%)
Squamous cell carcinoma			3 (5%)	
Subcutaneous tissue, fibroma	3 (6%)		3 (5%)	1 (2%)
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, lipoma	1 (2%)			
Subcutaneous tissue, sarcoma	1 (2%)			
Musculoskeletal System				
Bone	(3)	(7)		(10)
Carcinoma, metastatic, clitoral gland		1 (14%)		
Squamous cell carcinoma, metastatic, pharynx				1 (10%)
Skeletal muscle			(1)	(1)
Adenocarcinoma, metastatic, mammary gland				1 (100%)
Nervous System				
Brain	(49)	(35)	(64)	(50)
Astrocytoma malignant			1 (2%)	
Granular cell tumor benign			1 (2%)	
Oligodendroglioma malignant				1 (2%)
Squamous cell carcinoma, metastatic, pharynx				1 (2%)
Peripheral nerve				(1)
Schwannoma malignant				1 (100%)
Respiratory System				
Lung	(50)	(35)	(65)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	2 (6%)	8 (12%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple				1 (2%)
Alveolar/bronchiolar carcinoma			1 (2%)	
Basal cell carcinoma, metastatic, skin				1 (2%)
Carcinoma, metastatic	1 (2%)			
Carcinoma, metastatic, clitoral gland		1 (3%)		1 (2%)
Carcinoma, metastatic, Zymbal's gland			3 (5%)	
Histiocytic sarcoma			1 (2%)	1 (2%)
Sarcoma			1 (2%)	
Sarcoma, metastatic, uncertain primary site			1 (2%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Respiratory System (continued)				
Nose	(50)	(35)	(63)	(49)
Squamous cell carcinoma, metastatic, pharynx				1 (2%)
Special Senses System				
Harderian gland				(2)
Squamous cell carcinoma, metastatic, pharynx				1 (50%)
Zymbal's gland	(50)	(35)	(63)	(49)
Adenoma			2 (3%)	6 (12%)
Carcinoma		3 (9%)	17 (27%)	13 (27%)
Urinary System				
Kidney	(50)	(35)	(64)	(50)
Renal tubule, adenocarcinoma		1 (3%)		
Transitional epithelium, carcinoma	1 (2%)			
Urinary bladder	(49)	(35)	(64)	(49)
Transitional epithelium, papilloma				1 (2%)
Systemic Lesions				
Multiple organs[a]	(50)	(35)	(65)	(50)
Histiocytic sarcoma			1 (2%)	1 (2%)
Leukemia			1 (2%)	1 (2%)
Leukemia mononuclear	12 (24%)	13 (37%)	17 (26%)	4 (8%)
Lymphoma malignant histiocytic			1 (2%)	
Tumor Summary				
Total animals with primary neoplasms[b]	47	35	61	47
Total primary neoplasms	96	99	192	105
Total animals with benign neoplasms	41	31	50	32
Total benign neoplasms	70	64	104	54
Total animals with malignant neoplasms	22	25	52	42
Total malignant neoplasms	26	35	87	51
Total animals with secondary neoplasms[c]	1	1	5	5
Total secondary neoplasms	1	2	6	8
Total animals with malignant neoplasms of uncertain primary site			1	
Total animals with neoplasms uncertain-benign or malignant			1	
Total uncertain neoplasms			1	

[a] Number of animals with any tissue examined microscopically
[b] Primary tumors: all tumors except metastatic tumors
[c] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm

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

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 2 2 2	0 2 2 3	0 2 2 4	0 2 3 1	0 2 3 2	0 2 3 3	0 2 4 1	0 2 4 2	0 2 5 1	0 2 5 2	0 2 5 3	0 2 5 4	0 2 5 5	0 2 6 1	0 2 6 2	0 2 6 3	0 2 6 4	0 2 7 1	0 2 7 2	0 2 7 3	0 2 8 1	0 2 8 2	0 2 8 3	0 2 8 4	0 2 8 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery													+													4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	48
Schwannoma malignant										X																1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X	X	X	X				X				X	X	X	X		X				X			X	25
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma							X						X								X					6
C-cell, carcinoma						X					X											X				6
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm (continued)

Number of Days on Study	4	5	5	5	5	5	5	5	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7
	3	0	2	3	4	6	8	8	4	6	8	1	1	1	3	3	3	3	3	3	3	3	3	3
	1	5	4	3	7	2	7	9	1	3	3	1	5	5	3	3	3	3	3	4	4	4	4	4
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2	2	2
	8	0	6	3	1	4	7	0	3	4	4	2	0	7	9	9	9	9	0	0	1	1	1	2
	5	5	5	5	5	5	5	4	4	4	3	5	3	4	1	2	3	4	1	2	1	2	3	4	1
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+
Adenoma										X		X					X	X				X			
Carcinoma									X					X											
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal						X																			
Sarcoma stromal																		X							
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	M	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X													
Fibroadenoma											X	X	X	X	X		X	X	X	X	X	X			
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, sarcoma	X																								
Musculoskeletal System																									
Bone						+																			+
Nervous System																									
Brain	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 2 2 2	0 2 2 3	0 2 2 4	0 2 3 1	0 2 3 2	0 2 3 3	0 2 4 1	0 2 4 2	0 2 5 1	0 2 5 2	0 2 5 3	0 2 5 4	0 2 5 5	0 2 6 1	0 2 6 2	0 2 6 3	0 2 6 4	0 2 7 1	0 2 7 2	0 2 7 3	0 2 8 1	0 2 8 2	0 2 8 3	0 2 8 4	0 2 8 5	**Total Tissues/ Tumors**
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma								X																	X	7
Carcinoma						X							X													4
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal				X							X						X									4
Sarcoma stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	M	+	+	+	M	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	M	+	+	M	+	+	+	M	+	+	+	M	+	+	+	+	+	M	40
Thymoma benign									X																	1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																										1
Fibroadenoma				X	X									X						X		X				16
Fibroadenoma, multiple		X								X															X	3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																		X	X		X					3
Subcutaneous tissue, lipoma																									X	1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone														+												3
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																						X				1
Carcinoma, metastatic																						X				1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm (continued)

Number of Days on Study	4	5	5	5	5	5	5	5	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	3	0	2	3	4	6	8	8	4	6	8	1	1	1	3	3	3	3	3	3	3	3	3	3	3	3
	1	5	4	3	7	2	7	9	1	3	3	1	5	5	3	3	3	3	3	4	4	4	4	4	4	4
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2	2	2	2	2
	8	0	6	3	1	4	7	0	3	4	4	2	0	7	9	9	9	9	0	0	1	1	1	1	1	2
	5	5	5	5	5	5	5	4	4	4	3	5	3	4	1	2	3	4	1	2	1	2	3	4	1	
Special Senses System																										
Eye																										
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, carcinoma											X															
Urinary bladder	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X			X	X		X	X										X					

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 0 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 2 2 2	0 2 2 3	0 2 2 4	0 2 3 1	0 2 3 2	0 2 3 3	0 2 4 1	0 2 4 2	0 2 5 1	0 2 5 2	0 2 5 3	0 2 5 4	0 2 5 5	0 2 6 1	0 2 6 2	0 2 6 3	0 2 6 4	0 2 7 1	0 2 7 2	0 2 7 3	0 2 8 1	0 2 8 2	0 2 8 3	0 2 8 4	0 2 8 5	**Total Tissues/ Tumors**
Special Senses System																										
Eye				+												+					+					3
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X														X						X	X	X	12

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm

Number of Days on Study	4 1 1	4 1 1	4 6 2	5 0 0	5 2 3	5 3 8	5 6 7	5 8 2	6 0 7	6 1 4	6 3 8	6 3 8	6 4 1	6 5 8	6 5 8	6 6 2	6 6 3	6 6 5	6 7 7	7 0 8	7 1 5	7 2 6	7 3 3	7 3 3	7 3 3
Carcass ID Number	0 4 3 5	0 4 6 5	0 4 5 5	0 4 3 4	0 4 6 4	0 4 1 5	0 4 2 5	0 4 2 4	0 4 0 5	0 4 4 5	0 4 1 4	0 4 6 3	0 4 6 2	0 4 0 4	0 4 6 1	0 4 4 4	0 4 2 3	0 4 4 3	0 4 2 3	0 4 3 2	0 4 5 3	0 4 4 3	0 4 0 1	0 4 0 2	0 4 1 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																					X				
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx																									
Papilloma squamous																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue							+																		
Papilloma squamous							X																		
Tooth																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign	X																								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X			X						X	X	X		X	X	X				X			X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									X
Follicular cell, adenoma																									
Follicular cell, carcinoma																								X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 4 1 2	0 4 1 3	0 4 2 1	0 4 3 1	0 4 3 2	0 4 4 1	0 4 4 2	0 4 5 1	0 4 5 2	0 4 5 3	**Total Tissues/ Tumors**
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	35
Intestine large	+	+	+	+	+	+	+	+	+	+	35
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	35
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	35
Polyp adenomatous											1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	35
Intestine small	+	+	+	+	+	+	+	+	+	+	35
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	35
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	35
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	35
Liver	+	+	+	+	+	+	+	+	+	+	35
Mesentery							+				1
Pancreas	+	+	+	+	+	+	+	+	+	+	35
Pharynx									+		1
Papilloma squamous									X		1
Salivary glands	+	+	+	+	+	+	+	+	+	+	35
Stomach	+	+	+	+	+	+	+	+	+	+	35
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	35
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	35
Tongue	+										2
Papilloma squamous	X										2
Tooth									+		1
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	35
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	35
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	35
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	35
Pheochromocytoma benign			X				X				3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	35
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	34
Pituitary gland	+	+	+	+	+	+	+	+	+	+	35
Pars distalis, adenoma	X		X	X			X	X		X	17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	35
C-cell, adenoma						X					2
Follicular cell, adenoma			X								1
Follicular cell, carcinoma										X	2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	4 1 1	4 1 1	4 6 2	5 0 0	5 2 3	5 3 8	5 6 7	5 8 2	6 0 7	6 1 4	6 3 8	6 3 8	6 4 1	6 5 8	6 5 8	6 6 2	6 6 3	6 6 5	6 7 7	7 0 8	7 1 5	7 2 6	7 3 3	7 3 3	7 3 3
Carcass ID Number	0 4 3 5	0 4 6 5	0 4 5 5	0 4 3 4	0 4 6 4	0 4 1 5	0 4 2 5	0 4 2 4	0 4 0 5	0 4 4 5	0 4 1 4	0 4 6 3	0 4 6 2	0 4 0 4	0 4 6 1	0 4 4 4	0 4 2 3	0 4 4 3	0 4 2 2	0 4 3 3	0 4 5 4	0 4 0 3	0 4 0 1	0 4 0 2	0 4 1 1
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenoma						X				X	X						X		X		X				X
Carcinoma	X				X	X			X										X				X		
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal							X						X							X	X	X			
Polyp stromal, multiple									X																
Sarcoma stromal																		X							
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	M
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoacanthoma				X																					
Adenocarcinoma								X								X	X								
Adenoma																	X								
Fibroadenoma												X	X	X			X								
Fibroadenoma, multiple																								X	X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																	X								
Basal cell adenoma, multiple																									
Basal cell carcinoma										X															
Musculoskeletal System																									
Bone						+			+													+			
Carcinoma, metastatic, clitoral gland						X																			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 4 1 2	0 4 1 3	0 4 2 1	0 4 3 1	0 4 3 2	0 4 4 1	0 4 4 2	0 4 5 1	0 4 5 2	0 4 5 3	**Total Tissues/ Tumors**
General Body System											
None											
Genital System											
Clitoral gland	+	+	+	+	+	+	M	M	+	+	32
Adenoma									X	X	9
Carcinoma		X		X		X					9
Bilateral, adenoma	X										1
Ovary	+	+	+	+	+	+	+	+	+	+	35
Uterus	+	+	+	+	+	+	+	+	+	+	35
Polyp stromal	X										6
Polyp stromal, multiple				X							2
Sarcoma stromal				X							2
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	35
Lymph node	+	+	+	+	+	+	+	+	+	+	35
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	35
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	35
Spleen	+	+	+	+	+	+	+	+	+	+	35
Thymus	M	M	+	+	+	+	+	+	+	+	30
Integumentary System											
Mammary gland	+	+	+	+	+	+	+	+	+	+	35
Adenoacanthoma											1
Adenocarcinoma											3
Adenoma											1
Fibroadenoma					X	X	X	X	X	X	10
Fibroadenoma, multiple		X									3
Skin	+	+	+	+	+	+	+	+	+	+	35
Basal cell adenoma									X		2
Basal cell adenoma, multiple	X										1
Basal cell carcinoma											1
Musculoskeletal System											
Bone	+							+	+	+	7
Carcinoma, metastatic, clitoral gland											1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	4	4	4	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	
	1	1	6	0	2	3	6	8	0	1	3	3	4	5	5	6	6	6	7	0	1	2	3	3	
	1	1	2	0	3	8	7	2	7	4	8	8	1	8	8	2	3	5	7	8	5	6	3	3	
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
	3	6	5	3	6	1	2	2	0	4	1	6	6	0	6	4	2	4	2	3	5	0	0	0	1
	5	5	5	4	4	5	5	4	5	5	4	3	2	4	1	4	3	3	2	3	4	3	1	2	1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma		X																X							
Carcinoma, metastatic, clitoral gland						X																			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																						+			
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma	X		X																						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenocarcinoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear									X				X	X	X		X	X	X	X	X				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 150 ppm (continued)

Number of Days on Study	7 3 3	7 3 3	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 4 1 2	0 4 1 3	0 4 2 1	0 4 3 1	0 4 3 2	0 4 4 1	0 4 4 2	0 4 5 1	0 4 5 2	0 4 5 3	**Total Tissues/ Tumors**
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	35
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	35
Alveolar/bronchiolar adenoma											2
Carcinoma, metastatic, clitoral gland											1
Nose	+	+	+	+	+	+	+	+	+	+	35
Trachea	+	+	+	+	+	+	+	+	+	+	35
Special Senses System											
Eye		+									2
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	35
Carcinoma							X				3
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	35
Renal tubule, adenocarcinoma							X				1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	35
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear			X		X	X	X				13

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm

Number of Days on Study	0 5 3	0 8 7	2 8 5	3 4 4	4 0 0	4 0 3	4 4 8	4 5 4	4 7 7	4 8 7	4 9 1	4 9 4	4 9 6	5 1 5	5 1 7	5 2 1	5 2 3	5 3 0	5 3 1	5 3 2	5 3 8	5 4 5	5 4 5	5 4 4	5 6 7
Carcass ID Number	0 7 4 5	0 6 5 5	0 6 6 5	0 6 7 5	0 6 2 5	0 6 8 5	0 6 8 4	0 7 2 5	0 7 4 4	0 6 4 4	0 7 6 5	0 7 5 5	0 7 0 5	0 6 4 3	0 6 7 4	0 7 0 4	0 6 4 2	0 6 7 3	0 7 0 3	0 6 6 4	0 6 9 5	0 7 1 5	0 7 2 4	0 7 4 3	0 7 5 4
Alimentary System																									
Esophagus	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Liver	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma					X																				
Histiocytic sarcoma																									
Neoplastic nodule						X																			
Neoplastic nodule, multiple																								X	
Sarcoma, metastatic, uncertain primary site																									
Mesentery												+		+											
Pancreas	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx										+			+												
Papilloma squamous										X			X												
Squamous cell carcinoma																									
Salivary glands	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Stomach, glandular	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue							+											+	+						
Papilloma squamous																			X						
Squamous cell carcinoma							X											X							
Tooth																									
Peridontal tissue, histiocytic sarcoma																									
Cardiovascular System																									
Heart	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Adrenal gland, cortex	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	5 7 5	5 7 9	5 8 1	5 8 2	5 8 7	5 8 8	5 9 2	5 9 2	5 9 2	5 9 2	5 9 9	6 0 9	6 1 4	6 1 7	6 3 1	6 3 7	6 3 8	6 4 1	6 4 1	6 4 8	6 4 9	6 5 6	6 5 8	6 7 6	6 7 7
Carcass ID Number	0 6 6 3	0 6 6 2	0 7 0 2	0 7 5 3	0 7 1 4	0 7 3 5	0 6 7 2	0 7 1 3	0 7 6 3	0 7 6 4	0 7 3 4	0 6 8 3	0 7 0 1	0 6 4 1	0 6 9 4	0 7 6 2	0 7 3 3	0 6 9 3	0 7 1 2	0 7 6 1	0 7 8 2	0 6 8 2	0 7 3 3	0 7 2 2	0 7 2 1	0 6 5 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									X
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma															X			X			X				
Histiocytic sarcoma											X														
Neoplastic nodule			X					X	X				X		X								X		
Neoplastic nodule, multiple																									X
Sarcoma, metastatic, uncertain primary site																									
Mesentery												+													+
Pancreas	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx	+							+											+						
Papilloma squamous								X											X						
Squamous cell carcinoma	X																								
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue														+											
Papilloma squamous														X											
Squamous cell carcinoma																									
Tooth											+														
Peridontal tissue, histiocytic sarcoma											X														
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	6 8 0	6 9 3	6 9 3	6 9 4	7 0 9	7 1 5	7 1 5	7 1 5	7 1 5	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	
Carcass ID Number	0 6 7 1	0 6 6 1	0 7 3 2	0 7 4 2	0 6 8 1	0 6 9 2	0 7 1 1	0 7 5 1	0 7 5 2	0 6 5 1	0 6 6 2	0 6 5 3	0 6 9 1	0 7 3 1	0 7 4 1	**Total Tissues/ Tumors**
Alimentary System																
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Polyp adenomatous																1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Hepatocellular carcinoma						X			X							6
Histiocytic sarcoma																1
Neoplastic nodule				X				X	X			X				11
Neoplastic nodule, multiple			X				X									4
Sarcoma, metastatic, uncertain primary site	X															1
Mesentery																4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	62
Pharynx																5
Papilloma squamous																4
Squamous cell carcinoma																1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Sarcoma										X						1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Tongue																4
Papilloma squamous																2
Squamous cell carcinoma																2
Tooth																1
Peridontal tissue, histiocytic sarcoma																1
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Endocrine System																
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Pheochromocytoma benign									X							1
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Adenoma														X		1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	0 5 3	0 8 7	2 8 5	3 4 4	4 0 0	4 0 3	4 4 8	4 5 4	4 7 7	4 8 7	4 9 1	4 9 4	4 9 6	5 1 5	5 1 7	5 2 1	5 2 3	5 3 0	5 3 1	5 3 2	5 3 8	5 4 5	5 4 5	5 5 4	5 6 7
Carcass ID Number	0 7 4 5	0 6 5 5	0 6 6 5	0 6 7 5	0 6 2 5	0 6 8 5	0 6 8 4	0 7 2 5	0 7 4 4	0 6 4 4	0 7 6 5	0 7 5 5	0 7 0 5	0 6 4 3	0 6 7 4	0 7 0 4	0 6 4 2	0 6 7 3	0 7 0 3	0 6 6 4	0 6 9 5	0 7 1 5	0 7 2 4	0 7 4 3	0 7 5 4
Endocrine System (continued)																									
Adrenal gland, medulla	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign																X									
Islets, pancreatic	+		+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Parathyroid gland	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																					X				X
Thyroid gland	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
C-cell, carcinoma																									
Follicular cell, adenoma														X											
General Body System																									
Tissue NOS				+																					
Genital System																									
Clitoral gland	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X											X	X			X					
Carcinoma									X	X			X												X
Bilateral, carcinoma				X																					
Ovary	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor NOS																									
Periovarian tissue, sarcoma stromal, metastatic, uterus																									
Uterus	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Polyp stromal				X									X										X		
Polyp stromal, multiple																									
Sarcoma stromal																									
Hematopoietic System																									
Bone marrow	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, histiocytic sarcoma																									
Lymph node, mandibular	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Lymph node, mesenteric	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+		+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Thymus	+		+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	5 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 8 8 8 8 9 9 9 9 9 0 1 1 3 3 3 4 4 4 4 5 5 7 7 5 9 1 2 7 8 2 2 2 2 9 9 4 7 1 7 8 1 1 8 9 6 8 6 7
Carcass ID Number	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 6 6 7 7 7 7 6 7 7 7 7 6 7 6 6 7 7 6 7 7 6 7 7 7 6 6 6 0 5 1 3 7 1 6 6 3 8 0 4 9 6 3 9 1 6 8 2 2 2 5 3 2 2 3 4 5 2 3 3 4 4 3 1 1 4 2 3 3 2 1 2 3 2 1 4
Endocrine System (continued)	
Adrenal gland, medulla	+ +
Pheochromocytoma malignant	
Pheochromocytoma benign	X
Islets, pancreatic	+ +
Carcinoma	X
Parathyroid gland	+ + + + + + + + + + M + + + + + + + + + + M + + +
Pituitary gland	+ +
Pars distalis, adenoma	X X X X X X
Thyroid gland	+ +
C-cell, adenoma	X
C-cell, carcinoma	X
Follicular cell, adenoma	X
General Body System	
Tissue NOS	
Genital System	
Clitoral gland	+ + + + + + + + + + + + + + + + + + M + + + + M + +
Adenoma	X
Carcinoma	X X X X X X
Bilateral, carcinoma	X
Ovary	+ +
Granulosa cell tumor NOS	X
Periovarian tissue, sarcoma stromal, metastatic, uterus	
Uterus	+ +
Adenoma	X
Polyp stromal	X
Polyp stromal, multiple	X
Sarcoma stromal	X
Hematopoietic System	
Bone marrow	+ +
Lymph node	+ +
Mediastinal, histiocytic sarcoma	X
Lymph node, mandibular	+ +
Histiocytic sarcoma	X
Lymph node, mesenteric	+ +
Spleen	+ +
Histiocytic sarcoma	X
Thymus	+ + + + + + + + + + + + + + M + + + + + + + + + +

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	6 8 0	6 9 3	6 9 3	6 9 4	7 0 9	7 1 5	7 1 5	7 1 5	7 1 5	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	
Carcass ID Number	0 6 7 1	0 6 6 1	0 7 3 2	0 7 4 2	0 6 8 1	0 6 9 2	0 7 1 1	0 7 1 1	0 7 5 2	0 6 5 1	0 6 5 2	0 6 5 3	0 6 9 1	0 7 3 1	0 7 4 1	**Total Tissues/ Tumors**
Endocrine System (continued)																
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Pheochromocytoma malignant							X									1
Pheochromocytoma benign															X	3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Carcinoma																1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	62
Pituitary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	64
Pars distalis, adenoma	X	X			X			X				X	X			17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
C-cell, adenoma										X						2
C-cell, carcinoma														X		2
Follicular cell, adenoma												X				3
General Body System																
Tissue NOS																1
Genital System																
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	62
Adenoma		X							X		X		X	X		10
Carcinoma			X		X			X	X	X		X			X	17
Bilateral, carcinoma																2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Granulosa cell tumor NOS																1
Periovarian tissue, sarcoma stromal, metastatic, uterus										X						1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Adenoma																1
Polyp stromal				X		X			X							7
Polyp stromal, multiple																1
Sarcoma stromal										X						2
Hematopoietic System																
Bone marrow	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Mediastinal, histiocytic sarcoma																1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Histiocytic sarcoma																1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Histiocytic sarcoma																1
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	59

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	0 5 3	0 8 7	2 8 5	3 4 4	4 0 0	4 0 3	4 4 8	4 5 4	4 7 7	4 8 7	4 9 1	4 9 4	4 9 6	5 1 5	5 1 7	5 2 1	5 2 3	5 3 0	5 3 1	5 3 2	5 3 8	5 4 5	5 4 5	5 5 4	5 6 7
Carcass ID Number	0 7 4 5	0 6 5 5	0 6 6 5	0 6 7 5	0 6 2 5	0 6 8 5	0 6 8 4	0 7 2 5	0 7 4 4	0 6 4 4	0 7 6 5	0 7 5 5	0 7 0 5	0 6 4 3	0 6 7 4	0 7 0 4	0 6 4 2	0 6 7 3	0 7 0 3	0 6 6 4	0 6 9 5	0 7 1 5	0 7 2 4	0 7 4 3	0 7 5 4
Integumentary System																									
Mammary gland	+		+	M	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenocarcinoma			X																X						X
Adenoma																					X				
Fibroadenoma																					X				
Fibroadenoma, multiple																									
Sarcoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																X								X	
Basal cell carcinoma																									
Papilloma squamous																									
Squamous cell carcinoma																									
Subcutaneous tissue, fibroma																									X
Musculoskeletal System																									
Skeletal muscle				+																					
Nervous System																									
Brain	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant										X															
Granular cell tumor benign																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, zymbal's gland						X										X					X				
Histiocytic sarcoma																									
Sarcoma													X												
Sarcoma, metastatic, uncertain primary site																									
Nose	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	5 7 5	5 7 9	5 8 1	5 8 2	5 8 7	5 8 8	5 9 2	5 9 2	5 9 2	5 9 2	5 9 9	6 0 9	6 1 4	6 1 7	6 3 1	6 3 7	6 3 8	6 4 1	6 4 1	6 4 8	6 4 9	6 5 6	6 5 8	6 7 6	6 7 7
Carcass ID Number	0 6 6 3	0 6 6 2	0 7 0 2	0 7 5 3	0 7 1 4	0 7 3 5	0 6 7 2	0 7 1 3	0 7 6 3	0 7 6 4	0 7 3 4	0 6 8 3	0 7 0 1	0 6 4 1	0 6 9 4	0 7 6 2	0 7 3 3	0 6 9 3	0 7 1 2	0 7 6 1	0 6 8 2	0 7 2 3	0 7 2 2	0 7 2 1	0 6 5 4
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	M	+
Adenocarcinoma													X												X
Adenoma							X																		
Fibroadenoma							X		X	X															
Fibroadenoma, multiple																									X
Sarcoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma									X																
Basal cell carcinoma			X																						
Papilloma squamous													X												
Squamous cell carcinoma																									
Subcutaneous tissue, fibroma																			X						
Musculoskeletal System																									
Skeletal muscle																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									
Granular cell tumor benign																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X														X			X	X			
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, zymbal's gland																									
Histiocytic sarcoma											X														
Sarcoma																									
Sarcoma, metastatic, uncertain primary site																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	6 8 0	6 9 3	6 9 3	6 9 4	7 0 9	7 1 5	7 1 5	7 1 5	7 1 5	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	
Carcass ID Number	0 6 7 1	0 6 6 1	0 7 3 2	0 7 4 2	0 6 8 1	0 6 9 2	0 7 1 1	0 7 5 1	0 7 5 2	0 6 5 1	0 6 5 2	0 6 5 3	0 6 9 1	0 7 3 1	0 7 4 1	**Total Tissues/ Tumors**
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	58
Adenocarcinoma												X				6
Adenoma				X						X						4
Fibroadenoma	X		X				X								X	8
Fibroadenoma, multiple											X	X		X		4
Sarcoma									X							1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Basal cell adenoma		X													X	5
Basal cell carcinoma					X											2
Papilloma squamous																1
Squamous cell carcinoma								X	X				X			3
Subcutaneous tissue, fibroma		X														3
Musculoskeletal System																
Skeletal muscle																1
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Astrocytoma malignant																1
Granular cell tumor benign													X			1
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Alveolar/bronchiolar adenoma			X	X	X										X	8
Alveolar/bronchiolar carcinoma		X														1
Carcinoma, metastatic, zymbal's gland																3
Histiocytic sarcoma																1
Sarcoma																1
Sarcoma, metastatic, uncertain primary site	X															1
Nose	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	63
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	0 5 3	0 8 7	2 8 5	3 4 4	4 0 0	4 0 3	4 4 8	4 5 4	4 7 7	4 8 7	4 9 1	4 9 4	4 9 6	5 1 5	5 1 7	5 2 1	5 2 3	5 3 0	5 3 1	5 3 2	5 3 8	5 4 5	5 4 5	5 5 4	5 6 7
Carcass ID Number	0 7 4 5	0 6 5 5	0 6 6 5	0 6 7 5	0 6 2 5	0 6 8 5	0 6 8 4	0 7 2 5	0 7 4 4	0 6 4 4	0 7 6 5	0 7 5 5	0 7 0 5	0 6 4 3	0 6 7 4	0 7 0 4	0 6 4 2	0 6 7 3	0 7 0 3	0 6 6 4	0 6 9 5	0 7 1 5	0 7 2 4	0 7 4 3	0 7 5 4
Special Senses System																									
Eye	A				+		+																		
Zymbal's gland	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma						X	X				X				X	X				X	X		X		
Urinary System																									
Kidney	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Leukemia				X																					
Leukemia mononuclear														X											
Lymphoma malignant histiocytic																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	5 7 5	5 7 9	5 8 1	5 8 2	5 8 7	5 8 8	5 9 2	5 9 2	5 9 2	5 9 2	5 9 9	6 0 9	6 1 4	6 1 7	6 3 1	6 3 7	6 3 8	6 4 1	6 4 1	6 4 8	6 4 9	6 5 6	6 5 8	6 7 6	6 7 7
Carcass ID Number	0 6 6 3	0 6 6 2	0 7 0 2	0 7 5 3	0 7 1 4	0 7 3 5	0 6 7 2	0 7 1 3	0 7 6 3	0 6 6 4	0 7 3 4	0 6 8 3	0 7 0 1	0 6 4 1	0 6 9 4	0 6 6 2	0 7 3 3	0 6 9 3	0 7 1 2	0 7 6 1	0 6 8 2	0 7 2 3	0 7 2 2	0 7 2 1	0 6 5 4
Special Senses System																									
Eye	+																+								
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X														
Carcinoma		X				X									X	X	X	X							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma											X														
Leukemia																									
Leukemia mononuclear					X					X		X			X				X	X	X			X
Lymphoma malignant histiocytic																							X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 300 ppm (continued)

Number of Days on Study	6 8 0	6 9 3	6 9 3	6 9 4	7 0 9	7 1 5	7 1 5	7 1 5	7 1 5	7 3 3	7 3 3	7 3 3	7 3 3	7 3 3	7 3 4	
Carcass ID Number	0 6 7 1	0 6 6 1	0 7 3 2	0 7 4 2	0 6 8 1	0 6 9 2	0 7 1 1	0 7 5 1	0 7 5 2	0 6 5 1	0 6 5 2	0 6 5 3	0 6 9 1	0 7 3 1	0 7 4 1	**Total Tissues/ Tumors**
Special Senses System																
Eye					+											5
Zymbal's gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	63
Adenoma														X		2
Carcinoma					X		X							X		17
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Histiocytic sarcoma																1
Leukemia																1
Leukemia mononuclear		X	X			X	X	X					X	X	X	17
Lymphoma malignant histiocytic																1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 600 ppm

Number of Days on Study	1 8 6	2 2 9	2 5 8	2 7 6	3 3 9	3 4 4	3 4 9	3 5 1	3 5 5	3 5 5	3 7 2	3 7 9	3 8 3	3 8 4	3 8 4	3 9 2	4 0 0	4 0 3	4 0 3	4 1 1	4 1 1	4 1 1	4 1 1	4 1 2	4 1 3
Carcass ID Number	0 9 6 5	0 9 2 5	1 0 0 5	1 0 0 4	0 9 5 5	0 9 8 5	1 0 3 5	1 0 2 5	0 9 5 4	1 0 2 4	0 9 5 3	0 9 3 4	0 9 8 4	1 0 3 3	1 0 3 4	1 0 4 5	1 0 2 3	1 0 9 5	1 0 4 4	1 0 4 5	1 0 7 3	1 0 8 3	1 1 2 2	1 0 4 3	0 9 4 5	0 9 7 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, cystic, mucinous																									
Polyp adenomatous																									
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Polyp adenomatous											X														
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, cystic, mucinous																								X	
Polyp adenomatous																			X						
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Histiocytic sarcoma																								X	
Neoplastic nodule																				X			X		
Mesentery	+			+	+	+						+										+	+	+	
Schwannoma malignant																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx		+									+											+			
Papilloma squamous																									
Squamous cell carcinoma											X											X			
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue							+																		
Papilloma squamous							X																		
Tooth																									
Odontoma																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 600 ppm (continued)

Number of Days on Study	4 1 3	4 1 3	4 2 1	4 3 3	4 3 3	4 3 3	4 5 4	4 5 6	4 5 8	4 7 3	4 7 5	4 7 7	4 8 6	4 8 8	5 0 1	5 0 1	5 0 1	5 4 5	5 5 1	5 5 1	5 7 8	5 8 2	5 9 9	6 0 3	6 1 4	
Carcass ID Number	0 9 9 4	1 0 0 3	0 9 5 2	0 9 7 3	0 9 9 3	1 0 1 5	1 0 1 4	0 9 6 4	1 0 0 1	1 0 3 2	0 9 8 2	0 9 6 1	1 0 2 1	0 9 8 1	0 9 7 2	0 9 9 2	0 9 1 3	1 0 9 1	0 9 7 1	0 1 1 2	1 0 1 1	1 0 4 2	1 0 0 2	1 0 3 1	1 0 4 1	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	48
Adenocarcinoma, cystic, mucinous													X													1
Polyp adenomatous														X												1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp adenomatous																										1
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, cystic, mucinous																										1
Polyp adenomatous																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																X							X		X	3
Histiocytic sarcoma																										1
Neoplastic nodule														X			X				X				X	6
Mesentery		+						+						+												11
Schwannoma malignant								X																		1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pharynx															+											4
Papilloma squamous															X											1
Squamous cell carcinoma																										2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue	+		+							+																4
Papilloma squamous			X							X																3
Tooth		+										+														2
Odontoma		X										X														2
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 600 ppm (continued)

Number of Days on Study	1 8 1 6	2 2 9	2 5 8	2 7 6	3 3 9	3 4 4	3 4 9	3 5 1	3 5 5	3 5 5	3 7 2	3 7 9	3 8 3	3 8 4	3 8 4	3 9 2	4 0 0	4 0 3	4 0 3	4 1 1	4 1 1	4 1 1	4 1 1	4 1 2	4 1 3
Carcass ID Number	0 9 6 5	0 9 2 5	1 0 0 5	1 0 0 4	0 9 5 5	0 9 8 5	1 0 3 5	1 0 2 5	0 9 5 4	1 0 2 4	0 9 5 3	0 9 3 4	0 9 8 4	1 0 3 3	1 0 3 4	1 0 4 5	1 0 2 3	0 9 9 5	1 0 4 4	0 9 7 5	0 9 8 3	1 0 2 2	1 0 4 3	0 9 4 5	0 9 7 4
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X							X				X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																							X		
Follicular cell, carcinoma																					X				
General Body System																									
Tissue NOS			+																						
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X	X											X					
Carcinoma					X		X						X					X		X			X		X
Bilateral, carcinoma												X													
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								X	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, peripheral nerve			X																						
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								X	
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 600 ppm (continued)

Number of Days on Study	4 1 3	4 1 3	4 2 1	4 3 3	4 3 3	4 3 3	4 5 4	4 5 6	4 5 8	4 7 3	4 7 5	4 7 7	4 8 6	4 8 8	5 0 1	5 0 1	5 0 1	5 4 5	5 5 1	5 5 1	5 7 8	5 8 2	5 9 9	6 0 3	6 1 4	
Carcass ID Number	0 9 9 4	1 0 0 3	0 9 5 2	0 9 7 3	0 9 9 3	1 0 1 5	1 0 1 4	0 9 6 4	1 0 0 1	1 0 3 2	0 9 8 2	0 9 6 1	1 0 2 1	0 9 8 1	0 9 9 2	0 9 7 2	0 9 9 2	1 0 1 3	0 9 9 1	0 9 7 1	1 0 1 2	1 0 1 1	1 0 4 2	1 0 0 2	1 0 3 1	1 0 4 1	**Total Tissues/ Tumors**
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign								X																		1
Islets, pancreatic	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	47
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma								X																X		5
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma														X									X			3
Follicular cell, carcinoma																										1
General Body System																										
Tissue NOS									+																	2
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X	X			X				X		X				X	X								10
Carcinoma								X		X								X		X	X				X	13
Bilateral, carcinoma												X														2
Ovary	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Uterus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Polyp stromal								X												X						2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Schwannoma malignant, metastatic, peripheral nerve																										1
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Thymus	+	+	+	+	M	+	+	+	M	+	M	+	+	+	+	M	+	+	+	M	M	+	+	+	+	42

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 600 ppm (continued)

Number of Days on Study	1 8 6	2 2 9	2 5 8	2 7 6	3 3 9	3 4 4	3 4 9	3 5 1	3 5 5	3 5 5	3 7 2	3 7 9	3 8 3	3 8 4	3 8 4	3 9 2	4 0 0	4 0 3	4 0 3	4 1 1	4 1 1	4 1 1	4 1 1	4 1 2	4 1 3
Carcass ID Number	0 9 6 5	0 9 2 5	1 0 0 5	1 0 0 4	0 9 5 5	0 9 8 5	1 0 3 5	1 0 2 5	0 9 5 4	1 0 2 4	0 9 5 3	0 9 3 4	0 9 8 4	1 0 3 3	1 0 3 4	1 0 4 5	1 0 2 3	0 9 9 5	1 0 4 4	0 9 7 5	0 9 8 3	1 0 2 2	1 0 4 3	0 9 4 5	0 9 7 4
Integumentary System																									
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma								X													X				
Adenocarcinoma, multiple										X															
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Basal cell carcinoma						X																			
Keratoacanthoma																									
Papilloma squamous																		X							
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibrosarcoma																									
Musculoskeletal System																									
Bone																						+			
Squamous cell carcinoma, metastatic, pharynx																						X			
Skeletal muscle																					+				
Adenocarcinoma, metastatic, mammary gland																					X				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma malignant																									
Squamous cell carcinoma, metastatic, pharynx																						X			
Peripheral nerve			+																						
Schwannoma malignant			X																						
Spinal cord	+																								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar adenoma, multiple																									
Basal cell carcinoma, metastatic, skin																									
Carcinoma, metastatic, clitoral gland																		X							
Histiocytic sarcoma																								X	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic, pharynx																						X			
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 600 ppm (continued)

Number of Days on Study	4 1 3	4 1 3	4 2 1	4 3 3	4 3 3	4 3 3	4 5 4	4 5 6	4 5 8	4 7 3	4 7 5	4 7 7	4 8 6	4 8 8	5 0 1	5 0 1	5 0 1	5 4 5	5 5 1	5 5 1	5 7 8	5 8 2	5 9 9	6 0 3	6 1 4	
Carcass ID Number	0 9 9 4	1 0 0 3	0 9 5 2	0 9 7 3	0 9 9 3	1 0 1 5	1 0 1 4	0 9 6 4	1 0 0 1	1 0 3 2	0 9 8 2	0 9 6 1	1 0 2 1	0 9 8 1	0 9 7 2	0 9 9 2	1 0 1 3	0 9 9 1	0 9 7 1	0 0 1 2	1 0 1 1	1 0 4 2	1 0 0 2	1 0 3 1	1 0 4 1	**Total Tissues/ Tumors**
Integumentary System																										
Mammary gland	+	+	+	+	+	+	M	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	45
Adenocarcinoma																										2
Adenocarcinoma, multiple																										1
Fibroadenoma, multiple																								X		1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Basal cell adenoma							X						X				X									3
Basal cell carcinoma																						X				2
Keratoacanthoma										X																1
Papilloma squamous																										1
Subcutaneous tissue, fibroma																								X		1
Subcutaneous tissue, fibrosarcoma								X																		1
Musculoskeletal System																										
Bone					+				+		+	+	+				+		+	+			+			10
Squamous cell carcinoma, metastatic, pharynx																										1
Skeletal muscle																										1
Adenocarcinoma, metastatic, mammary gland																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma malignant					X																					1
Squamous cell carcinoma, metastatic, pharynx																										1
Peripheral nerve																										1
Schwannoma malignant																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma							X		X							X										3
Alveolar/bronchiolar adenoma, multiple																				X						1
Basal cell carcinoma, metastatic, skin																						X				1
Carcinoma, metastatic, clitoral gland																										1
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	49
Squamous cell carcinoma, metastatic, pharynx																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 600 ppm (continued)

Number of Days on Study	1 8 6	2 2 9	2 5 8	2 7 6	3 3 9	3 4 4	3 4 9	3 5 1	3 5 5	3 5 5	3 7 2	3 7 9	3 8 3	3 8 4	3 8 4	3 9 2	4 0 0	4 0 3	4 0 3	4 1 1	4 1 1	4 1 1	4 1 2	4 1 3
Carcass ID Number	0 9 6 5	0 9 2 5	1 0 0 5	1 0 0 4	0 9 5 5	0 9 8 5	1 0 3 5	1 0 2 5	0 9 5 4	1 0 2 4	0 9 5 4	0 9 3 3	0 9 8 4	0 9 3 4	0 0 3 4	0 0 4 5	0 0 2 3	1 9 9 5	0 0 4 4	1 9 7 5	0 9 8 3	1 0 2 2	1 0 4 3	0 9 4 5	0 9 7 4
Special Senses System																								
Eye				+																		+		
Harderian gland																						+		
Squamous cell carcinoma, metastatic, pharynx																						X		
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X	
Carcinoma									X				X	X	X	X			X					
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																								
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																							X	
Leukemia																								
Leukemia mononuclear		X		X																				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114: 600 ppm (continued)

Number of Days on Study	4 1 3	4 1 3	4 2 1	4 3 3	4 3 3	4 3 3	4 5 4	4 5 6	4 5 8	4 5 3	4 7 5	4 7 7	4 7 6	4 8 8	4 8 1	5 0 1	5 0 1	5 0 1	5 4 5	5 5 1	5 5 1	5 7 8	5 8 2	5 9 9	6 0 3	6 1 4	
Carcass ID Number	0 9 9 4	1 0 0 3	0 9 5 2	0 9 7 3	0 9 9 3	1 0 1 5	1 0 1 4	0 9 6 4	1 0 0 1	1 0 3 2	0 9 8 2	0 9 6 1	1 0 2 1	0 9 8 1	0 9 9 1	0 9 7 2	0 9 9 2	1 0 1 3	0 9 9 1	0 9 7 1	1 0 1 2	1 0 1 1	1 0 4 2	1 0 0 2	1 0 3 1	1 0 4 1	**Total Tissues/ Tumors**
Special Senses System																											
Eye																			+								3
Harderian gland																					+						2
Squamous cell carcinoma, metastatic, pharynx																											1
Zymbal's gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma											X	X			X						X					X	6
Carcinoma		X	X	X			X			X						X									X		13
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Transitional epithelium, papilloma										X																	1
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																											1
Leukemia														X													1
Leukemia mononuclear	X																								X		4

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

	0 ppm	150 ppm	300 ppm	600 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rates[a]	1/50 (2%)	3/35 (9%)	3/64 (5%)	1/50 (2%)
Effective rates[b]	1/50 (2%)	3/35 (9%)	3/59 (5%)	1/31 (3%)
Terminal rates[c]	0/36 (0%)	2/13 (15%)	1/6 (17%)	0/0 (0%)
First incidence (days)	641	411	521	456
Life table tests[d]	P=0.032	P=0.096	P=0.089	P=0.301
Logistic regression tests[d]	P=0.628	P=0.259	P=0.349	P=0.871N
Cochran-Armitage test[d]	P=0.569			
Fisher exact test[d]		P=0.187	P=0.374	P=0.622
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rates	1/50 (2%)	3/35 (9%)	5/64 (8%)	1/50 (2%)
Effective rates	1/50 (2%)	3/35 (9%)	5/59 (8%)	1/31 (3%)
Terminal rates	0/36 (0%)	2/13 (15%)	1/6 (17%)	0/0 (0%)
First incidence (days)	641	411	521	456
Life table tests	P=0.003	P=0.096	P=0.004	P=0.301
Logistic regression tests	P=0.306	P=0.259	P=0.060	P=0.871N
Cochran-Armitage test	P=0.484			
Fisher exact test		P=0.187	P=0.146	P=0.622
Clitoral Gland: Adenoma				
Overall rates	7/48 (15%)	10/32 (31%)	10/62 (16%)	10/50 (20%)
Effective rates	7/48 (15%)	10/32 (31%)	10/59 (17%)	10/43 (23%)
Terminal rates	5/34 (15%)	4/11 (36%)	3/6 (50%)	0/0 (0%)
First incidence (days)	663	538	403	351
Life table tests	P<0.001	P=0.004	P=0.002	P<0.001
Logistic regression tests	P=0.091	P=0.028	P=0.271	P=0.231
Cochran-Armitage test	P=0.327			
Fisher exact test		P=0.067	P=0.476	P=0.215
Clitoral Gland: Carcinoma				
Overall rates	4/48 (8%)	9/32 (28%)	19/62 (31%)	15/50 (30%)
Effective rates	4/48 (8%)	9/32 (28%)	19/60 (32%)	15/46 (33%)
Terminal rates	2/34 (6%)	4/11 (36%)	3/6 (50%)	0/0 (0%)
First incidence (days)	641	411	400	339
Life table tests	P<0.001	P=0.003	P<0.001	P<0.001
Logistic regression tests	P=0.003	P=0.030	P=0.001	P=0.022
Cochran-Armitage test	P=0.008			
Fisher exact test		P=0.021	P=0.003	P=0.003
Clitoral Gland: Adenoma or Carcinoma				
Overall rates	11/48 (23%)	17/32 (53%)	28/62 (45%)	23/50 (46%)
Effective rates	11/48 (23%)	17/32 (53%)	28/60 (47%)	23/46 (50%)
Terminal rates	7/34 (21%)	8/11 (73%)	6/6 (100%)	0/0 (0%)
First incidence (days)	641	411	400	339
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.003	P=0.001	P=0.014
Cochran-Armitage test	P=0.016			
Fisher exact test		P=0.006	P=0.009	P=0.006

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Intestine Large: Adenocarcinoma or Adenomatous Polyp				
Overall rates	0/50 (0%)	1/35 (3%)	0/65 (0%)	3/50 (6%)
Effective rates	0/50 (0%)	1/35 (3%)	0/61 (0%)	3/40 (8%)
Terminal rates	0/36 (0%)	0/13 (0%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–[e]	715	–	372
Life table tests	P<0.001	P=0.315	–	P=0.015
Logistic regression tests	P=0.098	P=0.369	–	P=0.364
Cochran-Armitage test	P=0.033			
Fisher exact test		P=0.412	–	P=0.084
Intestine Small: Adenocarcinoma or Adenomatous Polyp				
Overall rates	0/50 (0%)	0/35 (0%)	1/65 (2%)	2/50 (4%)
Effective rates	0/50 (0%)	0/35 (0%)	1/60 (2%)	2/33 (6%)
Terminal rates	0/36 (0%)	0/13 (0%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	–	677	403
Life table tests	P=0.007	–	P=0.318	P=0.136
Logistic regression tests	P=0.169	–	P=0.456	P=0.737
Cochran-Armitage test	P=0.040			
Fisher exact test		–	P=0.545	P=0.155
Liver: Neoplastic Nodule				
Overall rates	0/50 (0%)	0/35 (0%)	15/64 (23%)	6/50 (12%)
Effective rates	0/50 (0%)	0/35 (0%)	15/60 (25%)	6/33 (18%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	–	403	411
Life table tests	P<0.001	–	P<0.001	P<0.001
Logistic regression tests	P<0.001	–	P<0.001	P=0.009
Cochran-Armitage test	P<0.001			
Fisher exact test		–	P<0.001	P=0.003
Liver: Hepatocellular Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	6/64 (9%)	3/50 (6%)
Effective rates	0/50 (0%)	0/35 (0%)	6/61 (10%)	3/34 (9%)
Terminal rates	0/36 (0%)	0/13 (0%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	–	400	501
Life table tests	P<0.001	–	P<0.001	P<0.001
Logistic regression tests	P=0.003	–	P=0.025	P=0.009
Cochran-Armitage test	P=0.020			
Fisher exact test		–	P=0.025	P=0.063
Liver: Neoplastic Nodule or Hepatocellular Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	19/64 (30%)	8/50 (16%)
Effective rates	0/50 (0%)	0/35 (0%)	19/61 (31%)	8/34 (24%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	–	400	411
Life table tests	P<0.001	–	P<0.001	P<0.001
Logistic regression tests	P<0.001	–	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		–	P<0.001	P<0.001

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Lung: Alveolar/bronchiolar Adenoma				
Overall rates	1/50 (2%)	2/35 (6%)	8/65 (12%)	4/50 (8%)
Effective rates	1/50 (2%)	2/35 (6%)	8/59 (14%)	4/31 (13%)
Terminal rates	1/36 (3%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	733 (T)	411	582	454
Life table tests	P<0.001	P=0.263	P<0.001	P<0.001
Logistic regression tests	P=0.013	P=0.508	P=0.003	P=0.120
Cochran-Armitage test	P=0.034			
Fisher exact test		P=0.367	P=0.029	P=0.068
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rates	1/50 (2%)	2/35 (6%)	9/65 (14%)	4/50 (8%)
Effective rates	1/50 (2%)	2/35 (6%)	9/59 (15%)	4/31 (13%)
Terminal rates	1/36 (3%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	733 (T)	411	582	454
Life table tests	P<0.001	P=0.263	P<0.001	P<0.001
Logistic regression tests	P=0.007	P=0.508	P=0.001	P=0.120
Cochran-Armitage test	P=0.032			
Fisher exact test		P=0.367	P=0.016	P=0.068
Mammary Gland: Adenocarcinoma				
Overall rates	0/50 (0%)	3/35 (9%)	6/65 (9%)	3/50 (6%)
Effective rates	0/50 (0%)	3/35 (9%)	6/63 (10%)	3/46 (7%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	582	285	351
Life table tests	P<0.001	P=0.042	P=0.003	P=0.081
Logistic regression tests	P=0.456	P=0.089	P=0.061	P=0.755
Cochran-Armitage test	P=0.170			
Fisher exact test		P=0.066	P=0.027	P=0.106
Mammary Gland: Adenoma				
Overall rates	1/50 (2%)	1/35 (3%)	4/65 (6%)	0/50 (0%)
Effective rates	1/46 (2%)	1/30 (3%)	4/45 (9%)	0/8 (0%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	711	663	538	–
Life table tests	P=0.048	P=0.558	P=0.022	–
Logistic regression tests	P=0.433	P=0.672	P=0.166	P=0.999N
Cochran-Armitage test	P=0.363			
Fisher exact test		P=0.637	P=0.174	P=0.852N
Mammary Gland: Fibroadenoma				
Overall rates	19/50 (38%)	13/35 (37%)	12/65 (18%)	1/50 (2%)
Effective rates	19/46 (41%)	13/30 (43%)	12/45 (27%)	1/8 (13%)
Terminal rates	15/36 (42%)	9/13 (69%)	4/6 (67%)	0/0 (0%)
First incidence (days)	683	638	538	603
Life table tests	P<0.001	P=0.038	P=0.004	P=0.015
Logistic regression tests	P=0.394	P=0.194	P=0.466	P=0.641
Cochran-Armitage test	P=0.033N			
Fisher exact test		P=0.524	P=0.105N	P=0.121N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Oral Cavity (Pharynx and Tongue): Squamous Papilloma				
Overall rates	0/50 (0%)	3/35 (9%)	6/65 (9%)	4/50 (8%)
Effective rates	0/50 (0%)	3/35 (9%)	6/61 (10%)	4/44 (9%)
Terminal rates	0/36 (0%)	2/13 (15%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	567	487	349
Life table tests	P<0.001	P=0.023	P=0.011	P=0.005
Logistic regression tests	P=0.193	P=0.059	P=0.066	P=0.302
Cochran-Armitage test	P=0.076			
Fisher exact test		P=0.066	P=0.025	P=0.045
Oral Cavity (Pharynx and Tongue): Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	3/65 (5%)	2/50 (4%)
Effective rates	0/50 (0%)	0/35 (0%)	3/61 (5%)	2/40 (5%)
Terminal rates	0/36 (0%)	0/13 (0%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	–	454	372
Life table tests	P=0.016	–	P=0.129	P=0.167
Logistic regression tests	P=0.408	–	P=0.295	P=0.795
Cochran-Armitage test	P=0.077			
Fisher exact test		–	P=0.162	P=0.195
Oral Cavity (Pharynx and Tongue): Squamous Papilloma or Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	3/35 (9%)	9/65 (14%)	6/50 (12%)
Effective rates	0/50 (0%)	3/35 (9%)	9/61 (15%)	6/44 (14%)
Terminal rates	0/36 (0%)	2/13 (15%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	567	454	349
Life table tests	P<0.001	P=0.023	P=0.002	P=0.001
Logistic regression tests	P=0.156	P=0.059	P=0.025	P=0.225
Cochran-Armitage test	P=0.017			
Fisher exact test		P=0.066	P=0.003	P=0.009
Pharynx: Squamous Papilloma				
Overall rates	0/50 (0%)	1/35 (3%)	4/65 (6%)	1/50 (2%)
Effective rates	0/49 (0%)	1/32 (3%)	4/56 (7%)	1/12 (8%)
Terminal rates	0/36 (0%)	1/13 (8%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	733 (T)	487	501
Life table tests	P=0.008	P=0.298	P=0.048	P=0.207
Logistic regression tests	P=0.390	P=0.298	P=0.162	P=0.718
Cochran-Armitage test	P=0.063			
Fisher exact test		P=0.395	P=0.077	P=0.197
Pituitary Gland (Pars Distalis): Adenoma				
Overall rates	25/50 (50%)	17/35 (49%)	17/64 (27%)	5/49 (10%)
Effective rates	25/50 (50%)	17/35 (49%)	17/60 (28%)	5/41 (12%)
Terminal rates	19/36 (53%)	8/13 (62%)	2/6 (33%)	0/0 (0%)
First incidence (days)	524	411	538	355
Life table tests	P<0.001	P=0.046	P=0.008	P=0.016
Logistic regression tests	P=0.117N	P=0.505	P=0.290N	P=0.116N
Cochran-Armitage test	P<0.001N			
Fisher exact test		P=0.536N	P=0.016N	P<0.001N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Skin: Basal Cell Adenoma				
Overall rates	0/50 (0%)	3/35 (9%)	5/65 (8%)	3/50 (6%)
Effective rates	0/49 (0%)	3/33 (9%)	5/58 (9%)	3/19 (16%)
Terminal rates	0/36 (0%)	2/13 (15%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	663	521	454
Life table tests	P<0.001	P=0.016	P=0.005	P=0.006
Logistic regression tests	P=0.040	P=0.036	P=0.049	P=0.211
Cochran-Armitage test	P=0.018			
Fisher exact test		P=0.062	P=0.043	P=0.019
Skin: Basal Cell Adenoma or Carcinoma				
Overall rates	0/50 (0%)	4/35 (11%)	7/65 (11%)	5/50 (10%)
Effective rates	0/50 (0%)	4/35 (11%)	7/62 (11%)	5/45 (11%)
Terminal rates	0/36 (0%)	2/13 (15%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	614	521	344
Life table tests	P<0.001	P=0.006	P<0.001	P<0.001
Logistic regression tests	P=0.012	P=0.020	P=0.013	P=0.071
Cochran-Armitage test	P=0.058			
Fisher exact test		P=0.026	P=0.014	P=0.021
Skin: Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	3/65 (5%)	0/50 (0%)
Effective rates	0/38 (0%)	0/15 (0%)	3/10 (30%)	0/0 (0%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	–	715	–
Life table tests	P=0.003	–	P=0.002	–
Logistic regression tests	P=0.009	–	P=0.007	–
Cochran-Armitage test	P=0.004			
Fisher exact test		–	P=0.007	–
Skin: Squamous Papilloma or Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	4/65 (6%)	1/50 (2%)
Effective rates	0/50 (0%)	0/35 (0%)	4/60 (7%)	1/33 (3%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	–	614	403
Life table tests	P<0.001	–	P=0.001	P=0.417
Logistic regression tests	P=0.034	–	P=0.011	P=0.931
Cochran-Armitage test	P=0.168			
Fisher exact test		–	P=0.084	P=0.398
Skin (Subcutaneous Tissue): Fibroma				
Overall rates	3/50 (6%)	0/35 (0%)	3/65 (5%)	1/50 (2%)
Effective rates	3/44 (7%)	0/29 (0%)	3/41 (7%)	1/5 (20%)
Terminal rates	3/36 (8%)	0/13 (0%)	0/6 (0%)	0/0 (0%)
First incidence (days)	733 (T)	–	567	603
Life table tests	P=0.026	P=0.346N	P=0.168	P=0.015
Logistic regression tests	P=0.317	P=0.346N	P=0.540	P=0.381
Cochran-Armitage test	P=0.314			
Fisher exact test		P=0.213N	P=0.628	P=0.359

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rates	3/50 (6%)	0/35 (0%)	3/65 (5%)	2/50 (4%)
Effective rates	3/49 (6%)	0/33 (0%)	3/57 (5%)	2/18 (11%)
Terminal rates	3/36 (8%)	0/13 (0%)	0/6 (0%)	0/0 (0%)
First incidence (days)	733 (T)	–	567	456
Life table tests	P=0.003	P=0.346N	P=0.168	P=0.008
Logistic regression tests	P=0.204	P=0.346N	P=0.540	P=0.299
Cochran-Armitage test	P=0.296			
Fisher exact test		P=0.208N	P=0.586N	P=0.408
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma				
Overall rates	4/50 (8%)	0/35 (0%)	3/65 (5%)	2/50 (4%)
Effective rates	4/50 (8%)	0/33 (0%)	3/59 (5%)	2/22 (9%)
Terminal rates	3/36 (8%)	0/13 (0%)	0/6 (0%)	0/0 (0%)
First incidence (days)	431	–	567	456
Life table tests	P=0.029	P=0.219N	P=0.351	P=0.095
Logistic regression tests	P=0.621	P=0.089N	P=0.429N	P=0.736N
Cochran-Armitage test	P=0.512			
Fisher exact test		P=0.125N	P=0.408N	P=0.600
Thyroid Gland (C-cell): Adenoma				
Overall rates	6/50 (12%)	2/35 (6%)	2/64 (3%)	3/50 (6%)
Effective rates	6/50 (12%)	2/35 (6%)	2/59 (3%)	3/31 (10%)
Terminal rates	5/36 (14%)	2/13 (15%)	1/6 (17%)	0/0 (0%)
First incidence (days)	711	733 (T)	658	411
Life table tests	P=0.002	P=0.621N	P=0.447	P=0.003
Logistic regression tests	P=0.230	P=0.556N	P=0.606N	P=0.327
Cochran-Armitage test	P=0.370N			
Fisher exact test		P=0.280N	P=0.088N	P=0.525N
Thyroid Gland (C-cell): Carcinoma				
Overall rates	6/50 (12%)	0/35 (0%)	2/64 (3%)	0/50 (0%)
Effective rates	6/43 (14%)	0/27 (0%)	2/34 (6%)	0/3 (0%)
Terminal rates	4/36 (11%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	589	–	614	–
Life table tests	P=0.510N	P=0.117N	P=0.642	P=0.961N
Logistic regression tests	P=0.107N	P=0.051N	P=0.234N	P=0.297N
Cochran-Armitage test	P=0.132N			
Fisher exact test		P=0.046N	P=0.221N	P=0.651N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rates	12/50 (24%)	2/35 (6%)	4/64 (6%)	3/50 (6%)
Effective rates	12/50 (24%)	2/35 (6%)	4/59 (7%)	3/31 (10%)
Terminal rates	9/36 (25%)	2/13 (15%)	2/6 (33%)	0/0 (0%)
First incidence (days)	589	733 (T)	614	411
Life table tests	P=0.020	P=0.175N	P=0.439	P=0.006
Logistic regression tests	P=0.537N	P=0.064N	P=0.204N	P=0.725
Cochran-Armitage test	P=0.041N			
Fisher exact test		P=0.023N	P=0.011N	P=0.091N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Thyroid Gland (Follicular Cell): Adenoma				
Overall rates	0/50 (0%)	1/35 (3%)	3/64 (5%)	0/50 (0%)
Effective rates	0/48 (0%)	1/31 (3%)	3/52 (6%)	0/8 (0%)
Terminal rates	0/36 (0%)	1/13 (8%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	733 (T)	515	–
Life table tests	P=0.028	P=0.298	P=0.022	–
Logistic regression tests	P=0.246	P=0.298	P=0.124	–
Cochran-Armitage test	P=0.290			
Fisher exact test		P=0.392	P=0.137	–
Thyroid Gland (Follicular Cell): Carcinoma				
Overall rates	0/50 (0%)	2/35 (6%)	0/64 (0%)	1/50 (2%)
Effective rates	0/50 (0%)	2/35 (6%)	0/59 (0%)	1/31 (3%)
Terminal rates	0/36 (0%)	2/13 (15%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	733 (T)		411
Life table tests	P=0.067	P=0.058	–	P=0.405
Logistic regression tests	P=0.391	P=0.058	–	P=0.919
Cochran-Armitage test	P=0.457			
Fisher exact test		P=0.167	–	P=0.383
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rates	0/50 (0%)	3/35 (9%)	3/64 (5%)	1/50 (2%)
Effective rates	0/50 (0%)	3/35 (9%)	3/59 (5%)	1/31 (3%)
Terminal rates	0/36 (0%)	3/13 (23%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	733 (T)	515	411
Life table tests	P=0.002	P=0.011	P=0.022	P=0.405
Logistic regression tests	P=0.135	P=0.011	P=0.124	P=0.919
Cochran-Armitage test	P=0.398			
Fisher exact test		P=0.066	P=0.155	P=0.383
Tongue: Squamous Papilloma				
Overall rates	0/50 (0%)	2/35 (6%)	2/65 (3%)	3/50 (6%)
Effective rates	0/50 (0%)	2/35 (6%)	2/61 (3%)	3/44 (7%)
Terminal rates	0/36 (0%)	1/13 (8%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	567	531	349
Life table tests	P=0.001	P=0.102	P=0.192	P=0.034
Logistic regression tests	P=0.280	P=0.184	P=0.365	P=0.546
Cochran-Armitage test	P=0.104			
Fisher exact test		P=0.167	P=0.300	P=0.099
Tongue: Squamous Papilloma or Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	2/35 (6%)	4/65 (6%)	3/50 (6%)
Effective rates	0/50 (0%)	2/35 (6%)	4/61 (7%)	3/44 (7%)
Terminal rates	0/36 (0%)	1/13 (8%)	0/6 (0%)	0/0 (0%)
First incidence (days)	–	567	454	349
Life table tests	P=0.001	P=0.102	P=0.058	P=0.034
Logistic regression tests	P=0.386	P=0.184	P=0.187	P=0.546
Cochran-Armitage test	P=0.109			
Fisher exact test		P=0.167	P=0.087	P=0.099

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Uterus: Stromal Polyp				
Overall rates	4/50 (8%)	8/35 (23%)	8/65 (12%)	2/50 (4%)
Effective rates	4/50 (8%)	8/35 (23%)	8/61 (13%)	2/34 (6%)
Terminal rates	3/36 (8%)	2/13 (15%)	0/6 (0%)	0/0 (0%)
First incidence (days)	562	567	400	456
Life table tests	P<0.001	P=0.008	P=0.007	P=0.058
Logistic regression tests	P=0.490	P=0.041	P=0.276	P=0.761
Cochran-Armitage test	P=0.322N			
Fisher exact test		P=0.054	P=0.292	P=0.534N
Uterus: Stromal Sarcoma				
Overall rates	1/50 (2%)	2/35 (6%)	2/65 (3%)	0/50 (0%)
Effective rates	1/43 (2%)	2/27 (7%)	2/35 (6%)	0/3 (0%)
Terminal rates	1/36 (3%)	1/13 (8%)	1/6 (17%)	0/0 (0%)
First incidence (days)	733 (T)	665	588	–
Life table tests	P=0.131	P=0.197	P=0.136	–
Logistic regression tests	P=0.465	P=0.293	P=0.386	–
Cochran-Armitage test	P=0.509			
Fisher exact test		P=0.329	P=0.422	P=0.935N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rates	5/50 (10%)	9/35 (26%)	10/65 (15%)	2/50 (4%)
Effective rates	5/50 (10%)	9/35 (26%)	10/61 (16%)	2/34 (6%)
Terminal rates	4/36 (11%)	2/13 (15%)	1/6 (17%)	0/0 (0%)
First incidence (days)	562	567	400	456
Life table tests	P<0.001	P=0.006	P=0.001	P=0.058
Logistic regression tests	P=0.429	P=0.038	P=0.173	P=0.761
Cochran-Armitage test	P=0.254N			
Fisher exact test		P=0.053	P=0.243	P=0.404N
Zymbal's Gland: Adenoma				
Overall rates	0/50 (0%)	0/35 (0%)	2/65 (3%)	6/50 (12%)
Effective rates	0/50 (0%)	0/33 (0%)	2/59 (3%)	6/27 (22%)
Terminal rates	0/36 (0%)	0/13 (0%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	–	599	412
Life table tests	P<0.001	–	P=0.060	P<0.001
Logistic regression tests	P<0.001	–	P=0.191	P=0.007
Cochran-Armitage test	P<0.001			
Fisher exact test		–	P=0.291	P=0.001
Zymbal's Gland: Carcinoma				
Overall rates	0/50 (0%)	3/35 (9%)	17/65 (26%)	13/50 (26%)
Effective rates	0/50 (0%)	3/35 (9%)	17/61 (28%)	13/42 (31%)
Terminal rates	0/36 (0%)	1/13 (8%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	411	403	355
Life table tests	P<0.001	P=0.042	P<0.001	P<0.001
Logistic regression tests	P=0.010	P=0.163	P<0.001	P=0.021
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.066	P<0.001	P<0.001

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Zymbal's Gland: Adenoma or Carcinoma				
Overall rates	0/50 (0%)	3/35 (9%)	18/65 (28%)	19/50 (38%)
Effective rates	0/50 (0%)	3/35 (9%)	18/61 (30%)	19/42 (45%)
Terminal rates	0/36 (0%)	1/13 (8%)	1/6 (17%)	0/0 (0%)
First incidence (days)	–	411	403	355
Life table tests	P<0.001	P=0.042	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.163	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.066	P<0.001	P<0.001
All Organs: Mononuclear Cell Leukemia				
Overall rates	12/50 (24%)	13/35 (37%)	18/65 (28%)	5/50 (10%)
Effective rates	12/50 (24%)	13/35 (37%)	18/63 (29%)	5/49 (10%)
Terminal rates	7/36 (19%)	4/13 (31%)	3/6 (50%)	0/0 (0%)
First incidence (days)	533	607	344	229
Life table tests	P<0.001	P=0.012	P<0.001	P=0.007
Logistic regression tests	P=0.371	P=0.097	P=0.107	P=0.093N
Cochran-Armitage test	P=0.027N			
Fisher exact test		P=0.143	P=0.371	P=0.059N
All Organs: Benign Tumors				
Overall rates	41/50 (82%)	31/35 (89%)	50/65 (77%)	32/50 (64%)
Effective rates	41/50 (82%)	31/35 (89%)	50/61 (82%)	32/44 (73%)
Terminal rates	31/36 (86%)	12/13 (92%)	6/6 (100%)	0/0 (0%)
First incidence (days)	524	411	400	349
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.009	P=0.108	P=0.047	P=0.044
Cochran-Armitage test	P=0.103N			
Fisher exact test		P=0.305	P=0.598N	P=0.204N
All Organs: Malignant Tumors				
Overall rates	22/50 (44%)	25/35 (71%)	53/65 (82%)	42/50 (84%)
Effective rates	22/50 (44%)	25/35 (71%)	53/63 (84%)	42/49 (86%)
Terminal rates	14/36 (39%)	9/13 (69%)	5/6 (83%)	0/0 (0%)
First incidence (days)	431	411	285	229
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.019	P<0.001	P=0.130
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.011	P<0.001	P<0.001
All Organs: Benign or Malignant Tumors				
Overall rates	47/50 (94%)	35/35 (100%)	61/65 (94%)	47/50 (94%)
Effective rates	47/50 (94%)	35/35 (100%)	61/63 (97%)	47/49 (96%)
Terminal rates	34/36 (94%)	13/13 (100%)	6/6 (100%)	0/0 (0%)
First incidence (days)	431	411	285	229
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.066	P=0.157	P=0.142	P=0.431
Cochran-Armitage test	P=0.533			
Fisher exact test		P=0.198	P=0.391	P=0.510

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at first occurance of this tumor type in any of the groups
[c] Observed incidence at terminal kill
[d] Beneath the "0 ppm" are the P values associated with the trend test. Beneath the dosed group columns are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the effective incidence rates. For all tests, a negative trend or a lower incidence in a dosed group is indicated by N.
[e] Not applicable; no tumors in animal group

TABLE B4a
Historical Incidence of Skin Keratoacanthomas in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Overall Historical Incidence: Feed and Water	
Total	4/680 (0.6%)
Standard deviation	1.0%
Range	0%-2%

[a] Data as of 17 September 1990

TABLE B4b
Historical Incidence of Skin Basal Cell Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	2/680 (0.3%)	1/680 (0.1%)	3/680 (0.4%)
Standard deviation	0.8%	0.6%	0.7%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of 17 September 1990

TABLE B4c
Historical Incidence of Skin Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Squamous Cell Carcinoma
Overall Historical Incidence: Feed and Water			
Total	3/680 (0.4%)	2/680 (0.3%)	5/680 (0.7%)
Standard deviation	0.9%	0.8%	0.8%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of 17 September 1990

TABLE B4d
Historical Incidence of Zymbal's Gland Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	1/680 (0.1%)	5/680 (0.7%)	6/680 (0.9%)
Standard deviation	0.6%	1.0%	1.0%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of 17 September 1990

TABLE B4e
Historical Incidence of Clitoral Gland Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	56/606 (9.2%)	18/606 (3.0%)	74/606 (12.2%)
Standard deviation	4.4%	3.5%	5.7%
Range	2%-15%	0%-9%	5%-23%

[a] Data as of 17 September 1990

TABLE B4f
Historical Incidence of Liver Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	3/680 (0.4%)	0/680 (0.0%)	3/680 (0.4%)
Standard deviation	1.0%		1.0%
Range	0%-3%		0%-3%

[a] Data as of 17 September 1990

TABLE B4g
Historical Incidence of Lung Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Alveolar/bronchiolar Adenoma	Alveolar/bronchiolar Carcinoma	Alveolar/bronchiolar Adenoma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	10/679 (1.5%)	2/679 (0.3%)	13/679[b] (1.9%)
Standard deviation	1.6%	0.8%	2.1%
Range	0%-4%	0%-2%	0%-6%

[a] Data as of 17 September 1990
[b] Includes one carcinoma NOS and one adenosquamous carcinoma

TABLE B4h
Historical Incidence of Oral Cavity Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Papilloma or Squamous Cell Papilloma	Squamous Cell Carcinoma	Papilloma, Squamous Cell Papilloma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	4/680 (0.6%)	2/680 (0.3%)	6/680 (0.9%)
Standard deviation	1.0%	0.6%	1.0%
Range	0%-2%	0%-2%	0%-2%

[a] Data as of 17 September 1990; includes tongue, pharynx (palate), tooth (gingiva), and lip.

TABLE B4i
Historical Incidence of Mammary Gland Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls			
	Adenoma	Fibroadenoma	Adenocarcinoma	Adenoma, Fibroadenoma, or Adenocarcinoma
Overall Historical Incidence: Feed and Water				
Total	7/680 (1.0%)	235/680 (34.6%)	22/680 (3.2%)	255/680 (37.5%)
Standard deviation	1.3%	13.2%	4.0%	14.7%
Range	0%-4%	8%-56%	0%-12%	8%-60%

[a] Data as of 17 September 1990

TABLE B4j
Historical Incidence of Thyroid Gland Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Follicular Cell Adenoma	Follicular Cell Carcinoma	Follicular Cell Adenoma or Carcinoma
Overall Historical Incidence: Feed and Water			
Total	6/677 (0.9%)	2/677 (0.3%)	8/677 (1.2%)
Standard deviation	1.8%	0.8%	1.4%
Range	0%-6%	0%-3%	0%-6%

[a] Data as of 17 September 1990

TABLE B4k
Historical Incidence of Adrenal Medulla Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Benign Pheochromocytoma	Malignant Pheochromocytoma	Benign or Malignant Pheochromocytoma
Overall Historical Incidence: Feed and Water			
Total	40/661 (6.1%)	0/661 (0.0%)	40/661 (6.1%)
Standard deviation	3.7%		3.7%
Range	0%-14%		0%-14%

[a] Data as of 17 September 1990

TABLE B4l
Historical Incidence of Adenocarcinoma of the Intestine in Untreated Female F344/N Rats[a]

Study	Incidence in Controls	
	Small Intestine	Large Intestine
Overall Historical Incidence: Feed and Water		
Total	0/680 (0.0%)	0/680 (0.0%)

[a] Data as of 17 September 1990

TABLE B4m
Historical Incidence of Uterine Stromal Polyps in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Overall Historical Incidence: Feed and Water	
Total	120/680 (17.6%)
Standard deviation	5.6%
Range	8%-30%

[a] Data as of 17 September 1990

TABLE B4n
Historical Incidence of Leukemias in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Overall Historical Incidence: Feed and Water	
Total	170/680 (25.0%)
Standard deviation	6.3%
Range	14%-36%

[a] Data as of 17 September 1990

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

	0 ppm	150 ppm	300 ppm	600 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
Interim evaluations	20	10	10	20
Early deaths				
Natural death	4	6	17	13
Moribund	10	16	41	37
Accident death	0	0	1	0
Survivors				
Terminal sacrifice	36	13	6	0
Animals examined microscopically	50	35	65	50
Alimentary System				
Intestine large	(50)	(35)	(64)	(50)
Hemorrhage				1 (2%)
Intestine large, cecum	(50)	(35)	(63)	(49)
Edema		1 (3%)		
Hemorrhage				1 (2%)
Inflammation, acute		1 (3%)		
Ulcer			2 (3%)	1 (2%)
Artery, inflammation, chronic	1 (2%)			
Intestine large, colon	(50)	(35)	(63)	(48)
Parasite metazoan			3 (5%)	1 (2%)
Intestine large, rectum	(50)	(35)	(64)	(49)
Diverticulum				1 (2%)
Parasite metazoan	1 (2%)	3 (9%)	5 (8%)	
Intestine small, ileum	(50)	(35)	(63)	(50)
Parasite metazoan	1 (2%)		1 (2%)	1 (2%)
Liver	(50)	(35)	(64)	(50)
Angiectasis	1 (2%)	3 (9%)	6 (9%)	3 (6%)
Basophilic focus	38 (76%)	20 (57%)	38 (59%)	17 (34%)
Clear cell focus	1 (2%)			1 (2%)
Congestion			1 (2%)	
Cyst			3 (5%)	
Degeneration, cystic		5 (14%)	25 (39%)	14 (28%)
Eosinophilic focus		12 (34%)	38 (59%)	42 (84%)
Fatty change, diffuse			2 (3%)	
Fatty change, focal	1 (2%)			4 (8%)
Fatty change, multifocal		1 (3%)	6 (9%)	3 (6%)
Fibrosis, focal			1 (2%)	
Hematocyst		1 (3%)		1 (2%)
Hematopoietic cell proliferation		7 (20%)	15 (23%)	9 (18%)
Hemorrhage			1 (2%)	
Hepatodiaphragmatic nodule	9 (18%)	3 (9%)	5 (8%)	9 (18%)
Hyperplasia, focal			1 (2%)	
Infiltration cellular, polymorphonuclear			1 (2%)	
Inflammation, granulomatous, multifocal	22 (44%)	6 (17%)	2 (3%)	1 (2%)
Mixed cell focus	2 (4%)	9 (26%)	8 (13%)	15 (30%)
Necrosis, coagulative, multifocal	1 (2%)	1 (3%)	2 (3%)	4 (8%)
Necrosis, focal			1 (2%)	
Regeneration		2 (6%)	3 (5%)	1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Alimentary System (continued)				
Liver (continued)	(50)	(35)	(64)	(50)
Bile duct, cyst				1 (2%)
Bile duct, hyperplasia	10 (20%)	7 (20%)	4 (6%)	
Centrilobular, fatty change		3 (9%)	1 (2%)	1 (2%)
Centrilobular, necrosis, coagulative	1 (2%)	2 (6%)	6 (9%)	4 (8%)
Periportal, fatty change	1 (2%)		1 (2%)	
Mesentery	(4)	(1)	(4)	(11)
Inflammation, chronic		1 (100%)		
Fat, necrosis, focal	4 (100%)		2 (50%)	
Pancreas	(50)	(35)	(62)	(50)
Necrosis, focal				1 (2%)
Acinus, atrophy	10 (20%)	4 (11%)	6 (10%)	1 (2%)
Acinus, hyperplasia, focal			2 (3%)	
Salivary glands	(48)	(35)	(64)	(50)
Acinus, atrophy		1 (3%)		1 (2%)
Adventitia, inflammation, chronic			1 (2%)	
Stomach, forestomach	(50)	(35)	(64)	(50)
Hemorrhage				1 (2%)
Hyperplasia	1 (2%)		1 (2%)	2 (4%)
Inflammation, acute		2 (6%)	1 (2%)	
Inflammation, chronic	1 (2%)		1 (2%)	
Ulcer		1 (3%)	3 (5%)	2 (4%)
Stomach, glandular	(50)	(35)	(64)	(50)
Degeneration, cystic				1 (2%)
Edema				1 (2%)
Erosion			3 (5%)	4 (8%)
Mineralization	1 (2%)		1 (2%)	4 (8%)
Tooth		(1)	(1)	(2)
Peridontal tissue, hyperplasia		1 (100%)		
Cardiovascular System				
Heart	(50)	(35)	(64)	(49)
Cardiomyopathy	8 (16%)	5 (14%)	8 (13%)	2 (4%)
Mineralization	1 (2%)		1 (2%)	2 (4%)
Atrium, dilatation		1 (3%)		
Atrium, thrombus	1 (2%)	2 (6%)	6 (9%)	1 (2%)
Valve, inflammation, chronic active		1 (3%)		
Valve, thrombus		1 (3%)	2 (3%)	
Endocrine System				
Adrenal gland, cortex	(50)	(35)	(64)	(50)
Angiectasis	4 (8%)	5 (14%)	5 (8%)	8 (16%)
Atrophy		1 (3%)		
Fibrosis			1 (2%)	
Hematopoietic cell proliferation			1 (2%)	2 (4%)
Hemorrhage			1 (2%)	
Hyperplasia, focal			1 (2%)	
Necrosis, focal		1 (3%)		1 (2%)
Necrosis, multifocal	1 (2%)	1 (3%)		1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Endocrine System (continued)				
Adrenal gland, cortex (continued)	(50)	(35)	(64)	(50)
Vacuolization cytoplasmic, diffuse	1 (2%)	1 (3%)	2 (3%)	
Vacuolization cytoplasmic, focal	3 (6%)	4 (11%)	10 (16%)	6 (12%)
Vacuolization cytoplasmic, multifocal	1 (2%)	2 (6%)	3 (5%)	
Bilateral, necrosis, focal				1 (2%)
Bilateral, vacuolization cytoplasmic, focal			2 (3%)	
Bilateral, vacuolization cytoplasmic, multifocal				1 (2%)
Adrenal gland, medulla	(50)	(35)	(64)	(50)
Hyperplasia, focal	5 (10%)	5 (14%)	11 (17%)	2 (4%)
Hyperplasia, multifocal	1 (2%)	2 (6%)	4 (6%)	
Parathyroid gland	(48)	(34)	(62)	(47)
Hyperplasia				5 (11%)
Pituitary gland	(50)	(35)	(64)	(49)
Angiectasis	6 (12%)	2 (6%)	5 (8%)	
Congestion			1 (2%)	
Cyst	10 (20%)	8 (23%)	22 (34%)	7 (14%)
Hemorrhage	2 (4%)			
Pigmentation, hemosiderin				1 (2%)
Pars distalis, cyst				1 (2%)
Pars distalis, hyperplasia, focal		3 (9%)	1 (2%)	1 (2%)
Thyroid gland	(50)	(35)	(64)	(50)
Ultimobranchial cyst			1 (2%)	2 (4%)
C-cell, hyperplasia, focal	4 (8%)	3 (9%)	3 (5%)	1 (2%)
Follicle, cyst		1 (3%)	2 (3%)	
Follicular cell, hyperplasia, focal	1 (2%)	1 (3%)	3 (5%)	2 (4%)
General Body System				
Tissue NOS			(1)	(2)
Mineralization				1 (50%)
Genital System				
Clitoral gland	(48)	(32)	(62)	(50)
Hyperplasia, glandular, focal	4 (8%)	2 (6%)	8 (13%)	2 (4%)
Hyperplasia, squamous, focal	1 (2%)			
Inflammation, chronic active			1 (2%)	
Inflammation, suppurative			1 (2%)	1 (2%)
Duct, ectasia	1 (2%)	3 (9%)	3 (5%)	8 (16%)
Ovary	(50)	(35)	(64)	(49)
Bilateral, periovarian tissue, cyst				1 (2%)
Interstitium, cyst		1 (3%)		
Periovarian tissue, cyst	2 (4%)		1 (2%)	3 (6%)
Uterus	(50)	(35)	(64)	(49)
Decidual reaction			1 (2%)	
Hemorrhage		1 (3%)		
Hydrometra	2 (4%)	1 (3%)	2 (3%)	2 (4%)
Cervix, cyst	2 (4%)	1 (3%)		
Cervix, inflammation, suppurative	1 (2%)			
Endometrium, cyst	1 (2%)	2 (6%)	5 (8%)	
Endometrium, hyperplasia		1 (3%)		

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Hematopoietic System				
Bone marrow	(49)	(35)	(63)	(50)
Atrophy				4 (8%)
Hemorrhage			1 (2%)	1 (2%)
Hyperplasia, RE cell, multifocal	1 (2%)			
Myelofibrosis		2 (6%)		1 (2%)
Lymph node	(50)	(35)	(64)	(49)
Iliac, hyperplasia, plasma cell	1 (2%)			
Iliac, sinus, ectasia				1 (2%)
Inguinal, hyperplasia, plasma cell	1 (2%)			
Mediastinal, hemorrhage	10 (20%)	7 (20%)	7 (11%)	8 (16%)
Mediastinal, hyperplasia, plasma cell		1 (3%)		1 (2%)
Mediastinal, hyperplasia, RE cell			1 (2%)	
Pancreatic, hemorrhage			1 (2%)	
Lymph node, mandibular	(46)	(35)	(64)	(48)
Congestion	1 (2%)			1 (2%)
Hemorrhage	2 (4%)	2 (6%)	2 (3%)	5 (10%)
Hyperplasia, lymphoid				1 (2%)
Hyperplasia, plasma cell	9 (20%)	9 (26%)	23 (36%)	15 (31%)
Hyperplasia, RE cell			2 (3%)	
Adventitia, inflammation, chronic			1 (2%)	
Sinus, ectasia	6 (13%)	6 (17%)	3 (5%)	2 (4%)
Lymph node, mesenteric	(50)	(35)	(64)	(48)
Hemorrhage	1 (2%)	1 (3%)	4 (6%)	4 (8%)
Hyperplasia, RE cell			1 (2%)	
Infiltration cellular, mast cell				1 (2%)
Spleen	(50)	(35)	(63)	(50)
Depletion lymphoid				5 (10%)
Fibrosis		1 (3%)	2 (3%)	
Hematopoietic cell proliferation	3 (6%)	8 (23%)	20 (32%)	16 (32%)
Hyperplasia, RE cell, focal		1 (3%)		
Inflammation, granulomatous	1 (2%)			
Necrosis			1 (2%)	
Pigmentation, hemosiderin		1 (3%)	4 (6%)	4 (8%)
Capsule, hemorrhage			1 (2%)	
Red pulp, atrophy			2 (3%)	1 (2%)
Thymus	(40)	(30)	(59)	(42)
Ectopic parathyroid gland				1 (2%)
Hemorrhage			1 (2%)	1 (2%)
Epithelial cell, hyperplasia	25 (63%)	20 (67%)	29 (49%)	17 (40%)
Integumentary System				
Mammary gland	(49)	(35)	(58)	(45)
Galactocele		1 (3%)	2 (3%)	
Hyperplasia, nodular			1 (2%)	
Inflammation, chronic			1 (2%)	
Inflammation, suppurative	1 (2%)			
Duct, ectasia	6 (12%)	4 (11%)	3 (5%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Integumentary System (continued)				
Skin	(50)	(35)	(65)	(49)
Acanthosis, focal				1 (2%)
Inflammation, suppurative			1 (2%)	
Ulcer, multifocal			1 (2%)	
Hair follicle, hyperplasia, basal cell, focal		1 (3%)		
Subcutaneous tissue, inflammation, acute	1 (2%)			
Subcutaneous tissue, inflammation, chronic			1 (2%)	
Musculoskeletal System				
Bone	(3)	(7)		(10)
Cartilage, sternum, degeneration				1 (10%)
Cranium, fibrous osteodystrophy		1 (14%)		8 (80%)
Sternum, fibrous osteodystrophy		1 (14%)		2 (20%)
Sternum, osteopetrosis	3 (100%)	5 (71%)		1 (10%)
Nervous System				
Brain	(49)	(35)	(64)	(50)
Hemorrhage	1 (2%)	3 (9%)	6 (9%)	2 (4%)
Necrosis, focal			1 (2%)	
Brain stem, compression	12 (24%)	9 (26%)		1 (2%)
Meninges, inflammation, subacute			1 (2%)	
Respiratory System				
Lung	(50)	(35)	(65)	(50)
Congestion	1 (2%)	2 (6%)	2 (3%)	2 (4%)
Edema		1 (3%)	1 (2%)	
Hemorrhage		2 (6%)	4 (6%)	1 (2%)
Infiltration cellular, histiocytic	13 (26%)	22 (63%)	32 (49%)	27 (54%)
Mineralization				1 (2%)
Alveolar epithelium, hyperplasia, focal	6 (12%)	5 (14%)	13 (20%)	12 (24%)
Alveolar epithelium, hyperplasia, multifocal		1 (3%)	2 (3%)	8 (16%)
Bronchiole, foreign body			1 (2%)	
Bronchiole, inflammation, acute	1 (2%)		2 (3%)	
Interstitium, inflammation	1 (2%)	1 (3%)	1 (2%)	
Peribronchiolar, inflammation, chronic			1 (2%)	
Peribronchiolar, alveolus, inflammation, acute	1 (2%)		2 (3%)	
Nose	(50)	(35)	(63)	(49)
Fungus	2 (4%)		1 (2%)	
Hemorrhage		1 (3%)	1 (2%)	2 (4%)
Inflammation, suppurative	4 (8%)	2 (6%)	9 (14%)	3 (6%)
Trachea	(50)	(35)	(64)	(50)
Ectopic tissue			1 (2%)	
Inflammation, suppurative	1 (2%)			
Adventitia, inflammation, chronic			1 (2%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114 (continued)

	0 ppm	150 ppm	300 ppm	600 ppm
Special Senses System				
Eye	(3)	(2)	(5)	(3)
Cataract	3 (100%)	2 (100%)	3 (60%)	
Inflammation, chronic			1 (20%)	
Necrosis				1 (33%)
Anterior chamber, inflammation, suppurative			1 (20%)	1 (33%)
Retina, degeneration	2 (67%)		3 (60%)	
Zymbal's gland	(50)	(35)	(63)	(49)
Abscess				1 (2%)
Ectasia	1 (2%)			2 (4%)
Hyperplasia, glandular, focal	1 (2%)		2 (3%)	
Hyperplasia, squamous, focal			3 (5%)	3 (6%)
Hyperplasia, squamous, multifocal				1 (2%)
Inflammation, chronic active				1 (2%)
Urinary System				
Kidney	(50)	(35)	(64)	(50)
Cyst			2 (3%)	
Fatty change		1 (3%)		
Fibrosis, focal		1 (3%)		
Hydronephrosis				2 (4%)
Inflammation, suppurative			1 (2%)	
Karyomegaly				11 (22%)
Nephropathy	48 (96%)	32 (91%)	60 (94%)	48 (96%)
Pigmentation		1 (3%)	1 (2%)	
Interstitial tissue, fibrosis, focal		1 (3%)		
Interstitial tissue, inflammation		1 (3%)	1 (2%)	
Renal tubule, hyperplasia, focal				1 (2%)
Urinary bladder	(49)	(35)	(64)	(49)
Ectasia			2 (3%)	
Hemorrhage			2 (3%)	
Hyperplasia, focal	1 (2%)			

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA PROTOCOL

Testing was performed as reported by Mortelmans *et al.* (1986). C.I. Acid Red 114 was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strain (TA98, TA100, TA1535, TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C prior to the addition of soft agar supplemented with *l*-histidine and *d*-biotin and subsequent plating on minimal glucose agar plates. Incubation continued for an additional 48 hours.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of C.I. Acid Red 114. The high dose was limited to 10,000 μg/plate. All negative assays were repeated and all positive assays were repeated under the conditions which elicited the positive response.

A positive response in this assay is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants which was not dose-related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies occurs following chemical treatment.

PROTOCOL FOR THE *SALMONELLA* ASSAY WITH REDUCTIVE METABOLISM

Details of the experimental technique are presented in Reid *et al.* (1983, 1984a) and Prival and Mitchell (1982). Briefly, uncoded aliquots were obtained from Radian Corporation (Austin, TX). Overnight Difco nutrient broth cultures of *Salmonella typhimurium* TA1538 were used. The S9 fraction (metabolic activation enzymes and cofactors) was from Aroclor-induced male Fischer rat liver or noninduced female hamster liver. In the bacterial reduction system, C.I. Acid Red 114 was reduced overnight by incubation in brain-heart infusion broth with a washed suspension of rat cecal bacteria. Ethyl acetate extracts of the reduction mixtures were dissolved in dimethylsulfoxide (DMSO) and combined with TA1538 and rat liver S9 mix. This mixture was incubated with shaking for 20 minutes at 37° C. Top agar was then added, and the mixtures were plated onto minimal glucose agar plates. Incubation was continued for an additional 72 hours. For the flavin mononucleotide (FMN) reduction system, FMN was added to the DMSO solution containing the hamster liver S9 mix, TA1538, and the test chemical and incubated for 20 minutes at 37° C. The mixtures were then plated and incubated as described for the bacterial reduction system.

Each trial consisted of triplicate plates of the negative control and three doses of C.I. Acid Red 114. The positive control, 3,3′-dimethoxybenzidine, was tested at the same molar concentrations as C.I. Acid Red 114 for each test condition.

CHINESE HAMSTER OVARY CYTOGENETICS ASSAYS

Testing was performed as reported by Galloway *et al.* (1985, 1987) and presented briefly below. C.I. Acid Red 114 was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCE) and chromosomal aberrations (Abs) both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of test chemical; the high dose was limited by toxicity.

In the SCE test without S9, CHO cells were incubated for 26 hours with the study chemical in McCoy's 5A medium supplemented with 10% fetal bovine serum, *l*-glutamine (2mM), and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing the test chemical was removed and replaced with fresh medium plus BrdU and Colcemid, and incubated 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with the chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no test chemical, and incubated for an additional 26 hours with Colcemid present for the final 2 hours. Harvesting and staining was the same as for cells treated without S9.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with the study chemical for 8 to 10 hours; Colcemid was added and incubation continued for 2 to 3 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with the study chemical and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 10 hours in fresh medium with Colcemid present for the final 2 to 3 hours. Cells were harvested in the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were scored for frequency of SCE per cell from each dose level; 100 first-division metaphase cells were scored at each dose level for the Abs test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. Chromosomal aberration data are presented as percentage of cells with aberrations. As with SCE, both the dose-response curve and individual dose points were statistically analyzed. A statistically significant ($P<0.05$) difference for one dose point was considered weak evidence for a positive response (+w); significant differences for two or more doses indicated the trial was positive (+) (Galloway *et al.*, 1987).

DROSOPHILA PROTOCOL

The assays for induction of mutations were performed as described in Zimmering *et al.* (1985). C.I. Acid Red 114 was supplied as a coded aliquot from Radian Corporation (Austin, TX). Initially, C.I. Acid Red 114 was assayed in the sex-linked recessive lethal (SLRL) test by feeding the dye for 3 days to adult Canton-S wild-type males not older than 24 hours prior to treatment. Because no response was obtained, the chemical was retested by injection into adult males.

To administer a chemical by injection, a glass Pasteur pipette is drawn out in a flame to a microfine filament and the tip is broken off to allow delivery of the test solution. Injection is either done manually by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution to slightly distend the abdomen of the fly (0.2 to 0.3 μL) or by attaching the pipette to a microinjector which automatically delivers a calibrated volume. Flies are anesthetized with ether and immobilized on a strip of double stick tape; injection into the thorax under the wing is performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of C.I. Acid Red 114 at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. For the SLRL test, exposure by feeding was done by allowing Canton-S males (10-20 flies/vial) to feed for 72 hours on a solution of the study chemical in 5% sucrose. In the injection experiments, 24- to 72-hour-old Canton-S males were treated with a solution of the chemical dissolved in 0.7% saline or peanut oil and allowed to recover for 24 hours. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days; sample sperm from successive matings were treated at successively earlier post-meiotic stages. Heterozygous F_1 females were allowed to mate with their siblings and were then placed in individual vials. To identify clusters, F_1 daughters from the same parental male were kept together. A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event, and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution. If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as occurring in vials containing no wild-type males after 17 days; these were retested. A minimum of two experiments were performed for the study chemical, resulting in over 5,000 treated and 5,000 control chromosomes being tested. The second experiment was not performed if the results of the first experiment were clearly positive (induced frequency of recessive lethal mutations equal to or greater than 1%).

Recessive lethal data were analyzed by the normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered to be positive if the P value was less than 0.01 and the mutation frequency in the tested group was greater that 0.10%, or if the P value was less than 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if (a) the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15%, or (b) the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A result was considered to be negative if the P value was greater than 0.10 or if the frequency in the treatment group was less than 0.10%.

RESULTS

C.I. Acid Red 114 was tested for induction of gene mutations in *Salmonella typhimurium* using a standard preincubation protocol at concentrations of 100 to 10,000 μg/plate in the presence and the absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table 1; Mortelmans *et al.*, 1986). A weakly positive response was observed in TA98 with hamster S9 and an equivocal response was observed in TA100, also in the presence of hamster S9. No significant mutagenic activity was observed in TA1535 or TA1537. This compound, as with most benzidine congener dyes, requires reductive metabolism of the azo bonds to release the parent amine, which can then be oxidatively metabolized to an active mutagen. When tested under such a reductive/oxidative metabolism protocol, C.I. Acid Red 114 was highly mutagenic to *S. typhimurium* strain TA1538 (Table C2; Reid *et al.*, 1984a). Some mutagenic activity was observed in the presence of rat S9 without prior reduction, but the mutagenicity was greatly enhanced following reduction. With both reduction systems (cecal bacteria and FMN) the mutagenic response obtained with C.I. Acid Red 114 was greater than expected based on the activity of equimolar amounts of the parent diamine (dimethylbenzidine). This may indicate the presence of mutagenic impurities and the formation of additional reduction products in the crude dye mixture that was tested.

In cytogenetic tests with Chinese hamster ovary cells, C.I. Acid Red 114 did not induce sister chromatid exchanges (Table C3) or chromosomal aberrations (Table C4) when tested at concentrations up to 160 μg/mL, with or without Aroclor 1254-induced male Sprague-Dawley rat liver S9. Reductive metabolism was not used in these cytogenetic tests. No increase was observed in sex-linked recessive lethal mutations in germ cells of male *Drosophila* following administration of C.I. Acid Red 114 either by feeding (50,000 ppm) or by injection (1,500 ppm) (Table C5; Zimmering *et al.*, 1985).

TABLE C1
Mutagenicity of C.I. Acid Red 114 in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	78 ± 2.3	112 ± 1.2	88 ± 9.7	123 ± 11.5	82 ± 2.4	138 ± 2.0
	100	88 ± 9.8	113 ± 0.7	86 ± 6.2	121 ± 7.2	100 ± 7.6	162 ± 0.9
	333	75 ± 7.9	115 ± 1.5	97 ± 5.9	140 ± 5.7	108 ± 8.7	159 ± 19.2
	1,000	71 ± 7.8[c]	109 ± 9.5[c]	120 ± 5.5[c]	144 ± 14.8[c]	127 ± 2.6[c]	154 ± 18.4[c]
	3,333	60 ± 6.1[c]	47 ± 28.7[c]	139 ± 1.8[c]	153 ± 6.6[c]	98 ± 15.2[c]	135 ± 6.2[c]
	10,000	69 ± 9.6[c]	0 ± 0.0[c]	118 ± 9.9[c]	142 ± 11.8[c]	115 ± 3.9[c]	129 ± 6.3[c]
Trial summary		Negative	Negative	Weakly Positive	Equivocal	Equivocal	Negative
Positive control[d]		504 ± 66.9	1,203 ± 167.6	1,607 ± 185.5	3,138 ± 69.5	1,106 ± 63.6	2,398 ± 20.0
TA1535	0	7 ± 2.8	6 ± 0.7	6 ± 1.5	9 ± 0.6	13 ± 3.8	7 ± 2.3
	100	9 ± 0.3	5 ± 0.9	8 ± 1.2	10 ± 1.9	15 ± 1.2	10 ± 1.5
	333	8 ± 1.5	4 ± 0.7	7 ± 1.7	10 ± 3.2	12 ± 3.2	9 ± 2.3
	1,000	7 ± 1.5[c]	4 ± 1.7[c]	5 ± 1.9[c]	10 ± 0.9[c]	20 ± 2.1[c]	10 ± 0.9[c]
	3,333	5 ± 0.3[c]	2 ± 0.6[c]	12 ± 2.8[c]	4 ± 1.5[c]	19 ± 1.5[c]	7 ± 2.4[c]
	10,000	7 ± 0.9[c]	9 ± 4.0[c]	7 ± 0.9[c]	3 ± 0.5[c]	9 ± 0.6[c]	6 ± 0.6[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		615 ± 39.2	333 ± 80.3	83 ± 2.3	140 ± 14.0	79 ± 3.5	91 ± 18.7
TA1537	0	3 ± 0.3	1 ± 0.3	7 ± 2.1	5 ± 1.0	6 ± 1.5	4 ± 1.5
	100	4 ± 0.3	1 ± 0.3	5 ± 1.9	2 ± 0.7	4 ± 1.2	2 ± 0.6
	333	2 ± 0.6	1 ± 0.9	7 ± 0.7	2 ± 0.3	7 ± 1.3	4 ± 1.2
	1,000	2 ± 0.3[c]	1 ± 0.0[c]	8 ± 3.0[c]	2 ± 0.3[c]	6 ± 1.8[c]	2 ± 0.7[c]
	3,333	2 ± 0.9[c]	1 ± 0.3[c]	7 ± 1.7[c]	6 ± 1.0[c]	6 ± 2.3[c]	3 ± 0.7[c]
	10,000	2 ± 1.2[c]	1 ± 0.7[c]	5 ± 0.6[c]	3 ± 1.5[c]	4 ± 0.9[c]	1 ± 1.0[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		83 ± 8.5	286 ± 110.3	110 ± 9.8	54 ± 1.5	77 ± 3.0	65 ± 3.8
TA98	0	20 ± 1.5	6 ± 0.7				
	100	18 ± 1.3	8 ± 1.2				
	333	22 ± 3.8	5 ± 0.7				
	1,000	16 ± 0.3[c]	6 ± 0.9[c]				
	3,333	12 ± 0.9[c]	4 ± 1.7[c]				
	10,000	14 ± 1.8[c]	8 ± 2.1[c]				
Trial summary		Negative	Negative				
Positive control		671 ± 23.8	218 ± 35.3				

TABLE C1
Mutagenicity of C.I. Acid Red 114 in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate[b] +10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
TA98	0	27 ± 4.2	17 ± 0.9	20 ± 2.8	27 ± 2.4	21 ± 4.6	18 ± 2.3
(cont)	100	58 ± 1.9	21 ± 3.2	24 ± 1.8	29 ± 0.9	19 ± 1.2	26 ± 2.1
	333	75 ± 13.9	25 ± 4.1	33 ± 2.7	39 ± 0.9	27 ± 2.0	22 ± 3.0
	1,000	67 ± 7.5[c]	38 ± 4.1[c]	40 ± 1.8[c]	38 ± 1.2[c]	56 ± 3.2[c]	16 ± 1.8[c]
	3,333	33 ± 6.4[c]	27 ± 3.0[c]	26 ± 2.7[c]	31 ± 2.1[c]	30 ± 5.9[c]	13 ± 1.5[c]
	10,000	23 ± 1.2[c]	15 ± 6.0[c]	16 ± 1.7[c]	23 ± 2.0[c]	11 ± 2.4[c]	15 ± 2.2[c]
Trial summary		Positive	Weakly Positive	Weakly Positive	Negative	Equivocal	Negative
Positive control		716 ± 51.5	2,212 ± 8.4	969 ± 20.7	840 ± 78.6	1,776 ± 23.3	1,957 ± 55.0

[a] Study performed at Case Western Reserve University. The detailed protocol and these data are presented in Mortelmans *et al.* (1986). Cells and C.I. Acid Red 114 or solvent (distilled water) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. The solvent control is the 0 μg/plate dose.

[b] Revertants are presented as mean ± the standard error from three plates.

[c] Precipitate on plate

[d] 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE C2
Mutagenicity of C.I. Acid Red 114 in *Salmonella typhimurium* Strain TA1538 in Bacterial and Flavin Mononucleotide (FMN) Reduction Systems

Dose (μM)[a]	Reductive Metabolic System/Oxidative Metabolic System[a]		
	Bacterial reduction/rat S9[b]	No reduction/rat S9 Revertants/plate[d]	FMN reduction/hamster S9[c]
0.00	45	35	33
0.25	764 (105)	147 (129)	1,185 (169)
0.50	1,131 (131)	71 (139)	1,473 (241)
1.00	1,873 (123)	26 (179)	229 (278)

[a] For experiments with rat cecal bacterial reduction, C.I. Acid Red 114 was added to overnight incubation mixture. For experiments with the flavin mononucleotide reduction system, C.I. Acid Red 114 was added to the S9 mix.

[b] Overnight incubation with rat cecal bacteria followed by oxidative metabolism by Aroclor 1254-induced male Fischer rat liver S9 for 20 minutes and plating on minimal agar. Incubation was continued for 72 hours at 37° C, after which time revertant colonies were scored.

[c] FMN incorporated into the S9 mix during the 20-minute preincubation at 37° C. S9 was from noninduced female hamster livers. The mixtures were then plated, incubated, and scored as in [b].

[d] Revertants are presented as mean ± the standard error from three plates. Number of revertants obtained with the positive control, 3,3′-dimethoxybenzidine, at equimolar concentrations is given in parentheses after the values obtained for C.I. Acid Red 114. The detailed protocol and these data are presented by Reid *et al.* (1984a).

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by C.I. Acid Red 114[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosomes	SCEs/ Cell	Hrs in BrdU	Relative SCEs/Chromosome (%)[b]
-S9[c]								
Trial 1								
Summary: Positive								
Medium		50	1,039	398	0.38	8.0	26.0	
Mitomycin-C								
	0.0010	50	1,037	2,209	2.13	44.2	26.0	456.10
C.I. Acid Red 114								
	0.16	50	1,041	384	0.36	7.7	26.0	-3.71
	0.50	50	1,030	514	0.49	10.3	26.0	30.27*
	5.00	50	1,042	511	0.49	10.2	26.0	28.02*
	16.0	50	1,038	446	0.42	8.9	26.0	12.17
	50.0	50	1,035	483	0.46	9.7	26.0	21.82*
	160.0	0						
								P<0.001[d]
Trial 2								
Summary: Negative								
Medium		50	1,034	414	0.40	8.3	26.0	
Mitomycin-C								
	0.0050	50	1,047	1,430	1.36	28.6	26.0	241.13
	0.0100	50	1,046	1,854	1.77	37.1	26.0	342.69
C.I. Acid Red 114								
	10.0	50	1,037	455	0.43	9.1	26.0	9.59
	25.0	50	1,027	453	0.44	9.1	26.0	10.17
	50.0	50	1,032	456	0.44	9.1	26.0	10.36
	75.0	0						
	100.0	0						
								P=0.081

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by C.I. Acid Red 114 (continued)

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosomes	SCEs/ Cell	Hrs in BrdU	Relative SCEs/Chromosome (%)[b]
Trial 3								
Summary: Negative								
Medium		50	1,033	418	0.40	8.4	26.0	
Mitomycin-C	0.0100	50	1,035	2,148	2.07	43.0	26.0	412.89
C.I. Acid Red 114	0.5	50	1,043	423	0.40	8.5	26.0	0.23
	1.0	50	1,041	463	0.44	9.3	26.0	9.91
	5.0	50	1,036	449	0.43	9.0	26.0	7.11
	10.0	50	1,040	469	0.45	9.4	26.0	11.45
	25.0	50	1,030	442	0.42	8.8	26.0	6.05
	50.0	50	1,041	429	0.41	8.6	26.0	1.84
								P=0.232
+S9[e]								
Trial 1								
Summary: Negative								
Medium		50	1,036	502	0.48	10.0	26.0	
Cyclophosphamide	1.5	50	1,042	1,723	1.65	34.5	26.0	241.25
C.I. Acid Red 114	0.5	50	1,041	499	0.47	10.0	26.0	-1.08
	1.6	50	1,031	524	0.50	10.5	26.0	4.89
	5.0	50	1,034	507	0.49	10.1	26.0	1.19
	16.0	50	1,037	411	0.39	8.2	26.0	-18.21
	50.0	50	1,034	493	0.47	9.9	26.0	-1.60
	160.0	0					26.0	
								P=0.957

* Positive (≥20% increase over solvent control)

[a] Study performed at Environmental Health Research and Testing, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with C.I. Acid Red 114 or solvent (medium) as described in [c] and [d] below, and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.

[b] Percent increase in SCEs/chromosome of culture exposed to C.I. Acid Red 114 relative to those of culture exposed to solvent.

[c] In the absence of S9, cells were incubated with C.I. Acid Red 114 or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 hours.

[d] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose

[e] In the presence of S9, cells were incubated with C.I. Acid Red 114 or solvent for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with Colcemid present for the final 2 to 3 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

TABLE C4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by C.I. Acid Red 114[a]

-S9[b]					+S9[c]				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 – Harvest time: 12.0 hours Summary: Negative					**Trial 1** – Harvest time: 12.0 hours Summary: Negative				
Medium					Medium				
	100	0	0.00	0.0		100	0	0.00	0.0
Mitomycin-C					Cyclophosphamide				
0.50	100	52	0.52	31.0	25.0	100	48	0.48	31.0
C.I. Acid Red 114					C.I. Acid Red 114				
1.6	100	1	0.01	1.0	0.5	100	2	0.02	2.0
5.0	100	4	0.04	4.0	1.6	100	0	0.00	0.0
16.0	100	1	0.01	1.0	5.0	100	2	0.02	2.0
50.0	100	2	0.02	2.0	16.0	100	1	0.01	1.0
160.0	0				50.0	100	0	0.00	0.0
					160.0	0			
				P=0.158[d]					P=0.553
Trial 2 – Harvest time: 12.0 hours Summary: Negative									
Medium									
	100	0	0.00	0.0					
Mitomycin-C									
0.25	100	33	0.33	25.0					
C.I. Acid Red 114									
0.5	100	1	0.01	1.0					
1.0	100	1	0.01	1.0					
5.0	100	1	0.01	1.0					
10.0	100	0	0.00	0.0					
25.0	100	3	0.03	3.0					
50.0	100	1	0.01	1.0					
				P=0.127					

[a] Study performed at Environmental Health Research and Testing, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with C.I. Acid Red 114 or solvent (medium) as indicated in [b] and [c]. Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.

[b] In the absence of S9, cells were incubated with C.I. Acid Red 114 or solvent for 8 to 10 hours at 37° C. Cells were then washed and fresh medium containing Colcemid was added for an additional 2 to 3 hours followed by harvest.

[c] In the presence of S9, cells were incubated with C.I. Acid Red 114 compound or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 10 hours. Colcemid was added for the last 2 to 3 hours of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

[d] Significance of percent cells with Abs tested by the linear regression trend test vs. log of the dose

TABLE C5
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by C.I. Acid Red 114[a]

Route of Exposure	Dose (ppm)	Incidence of Deaths (percent)	Incidence of Sterility (percent)	No. of Lethals/No. of X Chromosomes Tested			
				Mating 1	Mating 2	Mating 3	Total[b]
Feeding	50,000	3	0	0/2,048	0/1,921	1/1,719	1/5,688 (0.02%)
	0			0/2,067	0/1,636	2/1,674	2/5,377 (0.04%)
Injection	1,500	22	14	0/2,402	3/2,056	3/1,667	6/6,125 (0.10%)
	0			1/2,209	1/2,015	3/1,807	5/6,031 (0.08%)

[a] Study performed at the University of Wisconsin-Madison. A detailed protocol of the sex-linked recessive lethal assay and these data are presented in Zimmering *et al.* (1985). In the feed exposure experiments, 24-hour-old Canton-S males were allowed to feed for 3 days on a solution of the C.I. Acid Red 114 dissolved in 5% sucrose. In the injection experiments, 24-hour-old Canton-S males were treated with a solution of C.I. Acid Red 114 dissolved in 0.7% saline and allowed to recover for 24 hours. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three broods of 3, 2, and 2 days; sample sperm from successive matings were treated as spermatozoa (mating 1), spermatids (mating 2), and spermatocytes (mating 3). F_1 heterozygous females were crossed to their siblings and placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters; clusters were removed in the injection experiment. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. Results were not significant at the 5% level (Margolin *et al.*, 1983).

[b] Combined total number of lethal mutations/number of X chromosomes tested for three mating trials.

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

TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Day Drinking Water Studies of C.I. Acid Red 114[a]

Organ	0 ppm	10,000 ppm	20,000 ppm	30,000 ppm
Male				
n	5	5	4	5
Necropsy body wt	218 ± 6	206 ± 2	181 ± 3**	168 ± 6**
Brain				
Absolute	1.76 ± 0.03	1.75 ± 0.02	1.74 ± 0.01	1.70 ± 0.02[b]
Relative	8.08 ± 0.13	8.52 ± 0.13*	9.61 ± 0.20**	10.33 ± 0.36**[b]
Heart				
Absolute	0.65 ± 0.02	0.63 ± 0.03	0.55 ± 0.02**	0.51 ± 0.01**
Relative	3.00 ± 0.07	3.05 ± 0.13	3.05 ± 0.08	3.06 ± 0.04
Liver				
Absolute	8.98 ± 0.31	9.38 ± 0.21	8.72 ± 0.22	8.29 ± 0.57[b]
Relative	41.20 ± 0.39	45.60 ± 0.74**	48.10 ± 0.47**	50.00 ± 1.05**[b]
Lung				
Absolute	1.00 ± 0.04	0.94 ± 0.02	0.83 ± 0.01**	0.79 ± 0.05**[b]
Relative	4.60 ± 0.10	4.58 ± 0.11	4.57 ± 0.11	4.80 ± 0.25[b]
R. Kidney				
Absolute	0.77 ± 0.02	0.85 ± 0.02	0.78 ± 0.04	0.78 ± 0.03
Relative	3.53 ± 0.09	4.14 ± 0.08**	4.31 ± 0.21**	4.65 ± 0.10**
R. Testis				
Absolute	1.10 ± 0.03	1.11 ± 0.04	1.13 ± 0.04	1.11 ± 0.05
Relative	5.07 ± 0.21	5.39 ± 0.15	6.23 ± 0.17**	6.62 ± 0.24**
Thymus				
Absolute	0.44 ± 0.02	0.40 ± 0.03	0.30 ± 0.01**	0.27 ± 0.03**
Relative	2.01 ± 0.09	1.96 ± 0.13	1.63 ± 0.02**	1.58 ± 0.14**
Female				
n	5	5	5	5
Necropsy body wt	151 ± 4	139 ± 2**	133 ± 3**	121 ± 3**
Brain				
Absolute	1.64 ± 0.01	1.63 ± 0.01	1.63 ± 0.02	1.60 ± 0.01*
Relative	10.90 ± 0.28	11.80 ± 0.12*	12.20 ± 0.22**	13.30 ± 0.34**
Heart				
Absolute	0.49 ± 0.01	0.48 ± 0.01	0.46 ± 0.01	0.42 ± 0.01**
Relative	3.25 ± 0.10	3.45 ± 0.07	3.47 ± 0.06	3.46 ± 0.05
Liver				
Absolute	5.82 ± 0.12	6.20 ± 0.11	5.85 ± 0.14	5.35 ± 0.22
Relative	38.60 ± 0.97	44.80 ± 0.36**	43.90 ± 0.41**	44.20 ± 0.76**
Lung				
Absolute	0.77 ± 0.03	0.76 ± 0.02	0.74 ± 0.02	0.64 ± 0.05*
Relative	5.12 ± 0.18	5.47 ± 0.06	5.59 ± 0.07	5.31 ± 0.39
R. Kidney				
Absolute	0.54 ± 0.01	0.58 ± 0.01*	0.59 ± 0.01**	0.57 ± 0.01
Relative	3.61 ± 0.10	4.22 ± 0.06**	4.46 ± 0.06**	4.69 ± 0.05**
Thymus				
Absolute	0.34 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.25 ± 0.01**
Relative	2.28 ± 0.04	2.41 ± 0.07	2.37 ± 0.13	2.05 ± 0.07

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=4

TABLE D2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Drinking Water Studies of C.I. Acid Red 114[a]

Organ	0 ppm	600 ppm	1,200 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	327 ± 4	321 ± 3	295 ± 5**	286 ± 6**	290 ± 5**	281 ± 4**
Brain						
Absolute	2.20 ± 0.08	2.00 ± 0.05*	2.02 ± 0.05	1.96 ± 0.10*	1.97 ± 0.05*	1.93 ± 0.04**
Relative	6.73 ± 0.22	6.23 ± 0.13	6.89 ± 0.22	6.84 ± 0.34	6.81 ± 0.22	6.88 ± 0.16
Heart						
Absolute	1.16 ± 0.07	1.00 ± 0.06	0.98 ± 0.06	1.04 ± 0.06	0.98 ± 0.07*	0.96 ± 0.06*
Relative	3.56 ± 0.22	3.12 ± 0.15	3.34 ± 0.21	3.64 ± 0.20	3.38 ± 0.21	3.40 ± 0.17
Liver						
Absolute	8.17 ± 0.13	8.73 ± 0.17	8.98 ± 0.27*	10.36 ± 0.26**	10.92 ± 0.34**	10.59 ± 0.25**
Relative	25.00 ± 0.24	27.20 ± 0.42**	30.40 ± 0.57**	36.30 ± 0.46**	37.60 ± 0.90**	37.70 ± 0.51**
Lung						
Absolute	1.34 ± 0.06	1.31 ± 0.06	1.22 ± 0.05[b]	1.36 ± 0.07	1.27 ± 0.06	1.19 ± 0.05
Relative	4.08 ± 0.15	4.07 ± 0.17	4.15 ± 0.18[b]	4.73 ± 0.19*	4.36 ± 0.17	4.23 ± 0.15
R. Kidney						
Absolute	1.22 ± 0.06	1.04 ± 0.03*	1.12 ± 0.06	1.09 ± 0.05	1.13 ± 0.04	1.08 ± 0.03
Relative	3.72 ± 0.17	3.25 ± 0.09	3.83 ± 0.22	3.80 ± 0.18	3.92 ± 0.15	3.85 ± 0.05
R. Testis						
Absolute	1.66 ± 0.06	1.46 ± 0.02	1.55 ± 0.06	1.62 ± 0.07	1.53 ± 0.06	1.44 ± 0.05*
Relative	5.08 ± 0.18	4.55 ± 0.04	5.27 ± 0.24	5.65 ± 0.23	5.27 ± 0.21	5.13 ± 0.18
Thymus						
Absolute	0.44 ± 0.06	0.36 ± 0.05	0.33 ± 0.05	0.44 ± 0.06	0.39 ± 0.06	0.34 ± 0.05
Relative	1.34 ± 0.19	1.11 ± 0.15	1.13 ± 0.18	1.52 ± 0.21	1.36 ± 0.22	1.20 ± 0.17
Female						
n	10	10	10	10	10	10
Necropsy body wt	183 ± 3	176 ± 2	171 ± 2*	174 ± 3*	171 ± 2**	165 ± 2**
Brain						
Absolute	1.79 ± 0.02	1.80 ± 0.01	1.78 ± 0.01	1.78 ± 0.01	1.79 ± 0.02	1.78 ± 0.01
Relative	9.84 ± 0.15	10.28 ± 0.11*	10.42 ± 0.18**	10.29 ± 0.15*	10.48 ± 0.08**	10.80 ± 0.10**
Heart						
Absolute	0.62 ± 0.01	0.60 ± 0.01	0.61 ± 0.01	0.62 ± 0.01	0.61 ± 0.02	0.58 ± 0.01
Relative	3.37 ± 0.07	3.42 ± 0.06	3.55 ± 0.07	3.54 ± 0.05	3.56 ± 0.09	3.51 ± 0.03
Liver						
Absolute	4.62 ± 0.08	5.01 ± 0.15*	5.12 ± 0.07**	5.71 ± 0.09**	5.54 ± 0.09**	5.77 ± 0.17**
Relative	25.30 ± 0.28	28.60 ± 1.02**	30.00 ± 0.27**	32.90 ± 0.31**	32.40 ± 0.26**	35.00 ± 1.02**
Lung						
Absolute	0.90 ± 0.02	0.94 ± 0.02	0.91 ± 0.01	0.92 ± 0.02[b]	0.89 ± 0.02	0.85 ± 0.02
Relative	4.94 ± 0.10	5.34 ± 0.13*	5.30 ± 0.07*	5.26 ± 0.09[b]	5.19 ± 0.08	5.19 ± 0.12
R. Kidney						
Absolute	0.61 ± 0.01	0.62 ± 0.01	0.65 ± 0.02	0.68 ± 0.01	0.67 ± 0.01	0.75 ± 0.05**
Relative	3.32 ± 0.07	3.53 ± 0.09	3.82 ± 0.10**	3.91 ± 0.07**	3.90 ± 0.07**	4.57 ± 0.29**
Thymus						
Absolute	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.21 ± 0.01*	0.20 ± 0.01*	0.18 ± 0.01**
Relative	1.32 ± 0.05	1.32 ± 0.06	1.31 ± 0.05	1.21 ± 0.04	1.20 ± 0.06	1.07 ± 0.06**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE D3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 9-Month Interim Sacrifice in the 2-Year Drinking Water Studies of C.I. Acid Red 114[a]

Organ	Male		Female	
	0 ppm	300 ppm	0 ppm	600 ppm
n	10	10	10	10
Necropsy body wt	426 ± 6	436 ± 5	247 ± 5	230 ± 4*
Brain				
Absolute	2.06 ± 0.02	2.07 ± 0.02	1.85 ± 0.01	1.89 ± 0.02
Relative	4.84 ± 0.07	4.76 ± 0.06	7.52 ± 0.15	8.25 ± 0.15**
Liver				
Absolute	9.94 ± 0.18	12.30 ± 0.47**	5.80 ± 0.12	6.88 ± 0.19**
Relative	23.30 ± 0.32	28.20 ± 1.04**	23.60 ± 0.33	30.00 ± 0.98**
R. Kidney				
Absolute	2.42 ± 0.04	2.58 ± 0.05*	1.45 ± 0.02	1.58 ± 0.03**
Relative	5.67 ± 0.08	5.93 ± 0.10	5.89 ± 0.12	6.90 ± 0.17**

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

TABLE D4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Sacrifice in the 2-Year Drinking Water Studies of C.I. Acid Red 114[a]

Organ	0 ppm	70 ppm	150 ppm	300 ppm
Male				
n	10	10	10	10
Necropsy body wt	446 ± 6	437 ± 6	451 ± 6	434 ± 5
Brain				
Absolute	2.11 ± 0.03	2.10 ± 0.02	2.14 ± 0.03	2.13 ± 0.02
Relative	4.72 ± 0.05	4.80 ± 0.03	4.75 ± 0.09	4.91 ± 0.06*
Liver				
Absolute	10.85 ± 0.20	11.59 ± 0.42	11.65 ± 0.23	12.47 ± 0.43**
Relative	24.40 ± 0.62	26.50 ± 0.95	25.80 ± 0.48	28.70 ± 0.80**
R. Kidney				
Absolute	2.61 ± 0.05	2.64 ± 0.05	2.63 ± 0.08	2.67 ± 0.06
Relative	5.86 ± 0.09	6.05 ± 0.13	5.83 ± 0.18	6.16 ± 0.09

Organ	0 ppm	150 ppm	300 ppm	600 ppm
Female				
n	10	9	9	10
Necropsy body wt	275 ± 4	298 ± 5	280 ± 8	243 ± 10**
Brain				
Absolute	1.94 ± 0.02	1.92 ± 0.02	1.91 ± 0.02	1.91 ± 0.02
Relative	7.06 ± 0.09	6.47 ± 0.10	6.86 ± 0.19	7.97 ± 0.31**
Liver				
Absolute	6.09 ± 0.13	7.92 ± 0.27**	8.57 ± 0.38**	12.09 ± 0.54**
Relative	22.20 ± 0.36	26.60 ± 0.76	30.70 ± 1.48**	50.40 ± 2.54**
R. Kidney				
Absolute	1.59 ± 0.03	1.77 ± 0.04*	1.87 ± 0.06**	2.31 ± 0.09**
Relative	5.78 ± 0.11	5.95 ± 0.11	6.68 ± 0.15*	9.64 ± 0.45**

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

APPENDIX E
HEMATOLOGY, CLINICAL CHEMISTRY, AND URINALYSIS RESULTS

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Drinking Water Studies of C.I. Acid Red 114[a]

Analysis	0 ppm	600 ppm	1,200 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	48.7 ± 0.7	48.7 ± 0.5	50.2 ± 0.6	49.5 ± 0.7	47.2 ± 0.6	46.4 ± 0.8
Hemoglobin (g/dL)	16.6 ± 0.2	16.4 ± 0.2	16.5 ± 0.2	16.1 ± 0.2	15.9 ± 0.1**	16.2 ± 0.2*
Erythrocytes ($10^6/\mu L$)	9.34 ± 0.11	9.37 ± 0.10	9.26 ± 0.11	9.00 ± 0.12*	8.81 ± 0.08**	8.71 ± 0.12**
Leukocytes ($10^3/\mu L$)	5.13 ± 0.30	4.63 ± 0.18	5.06 ± 0.21	5.00 ± 0.19	5.44 ± 0.15	6.17 ± 0.34*
Segmented neutrophils ($10^3/\mu L$)	1.53 ± 0.09	1.35 ± 0.13	1.26 ± 0.12	1.08 ± 0.08**	0.93 ± 0.09**	0.84 ± 0.10**
Lymphocytes ($10^3/\mu L$)	3.55 ± 0.29	3.23 ± 0.15	3.76 ± 0.23	3.90 ± 0.17	4.48 ± 0.16*	5.31 ± 0.32**
Monocytes ($10^3/\mu L$)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Eosinophils ($10^3/\mu L$)	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
n	10	10	10	10	10	10
Clinical chemistry						
Urea nitrogen (mg/dL)	17.70 ± 0.54	17.10 ± 0.99	16.30 ± 0.50	19.00 ± 0.76	19.40 ± 0.73	21.40 ± 0.37**
Creatinine (mg/dL)	0.61 ± 0.02	0.57 ± 0.02	0.53 ± 0.02	0.54 ± 0.02	0.63 ± 0.02	0.67 ± 0.02
Alanine aminotransferase (IU/L)	33 ± 2[b]	32 ± 2[b]	43 ± 4	38 ± 1	43 ± 2**	41 ± 2*[b]
Lactate dehydrogenase (IU/L)	466 ± 82	758 ± 56*	882 ± 59**	719 ± 79**	970 ± 44**	1038 ± 89**
Sorbitol dehydrogenase (IU/L)	7 ± 1[b]	10 ± 1**[b]	17 ± 2**	15 ± 1**	23 ± 1**	20 ± 2**[b]

TABLE E1
Hematology and Clinical Chemistry Data for Rats in the 13-Week Drinking Water Studies of C.I. Acid Red 114 (continued)

Analysis	0 ppm	600 ppm	1,200 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	47.1 ± 0.5	45.4 ± 0.6	42.1 ± 0.5**	36.6 ± 0.5**	36.4 ± 0.6**	36.4 ± 0.8**
Hemoglobin (g/dL)	16.6 ± 0.2	15.9 ± 0.2*	15.3 ± 0.2**	14.8 ± 0.2**	14.7 ± 0.2**	14.7 ± 0.2**
Erythrocytes ($10^6/\mu L$)	8.75 ± 0.09	8.09 ± 0.10**	7.32 ± 0.08**	6.32 ± 0.09**	6.28 ± 0.12**	6.32 ± 0.15**
Leukocytes ($10^3/\mu L$)	5.53 ± 0.50	5.75 ± 0.49	6.14 ± 0.50	6.43 ± 0.47	6.17 ± 0.39	6.37 ± 0.20
Segmented neutrophils ($10^3/\mu L$)	0.85 ± 0.04	0.83 ± 0.10	0.70 ± 0.07	0.66 ± 0.06	0.74 ± 0.10	0.78 ± 0.08
Lymphocytes ($10^3/\mu L$)	4.62 ± 0.49	4.89 ± 0.40	5.42 ± 0.48	5.73 ± 0.43	5.41 ± 0.41	5.55 ± 0.18
Monocytes ($10^3/\mu L$)	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Eosinophils ($10^3/\mu L$)	0.05 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
n	10	10	10	9	10	10
Clinical chemistry						
Urea nitrogen (mg/dL)	17.10 ± 0.28	17.00 ± 0.58	16.78 ± 0.52[b]	17.56 ± 0.29	16.20 ± 1.15	18.10 ± 0.57
Creatinine (mg/dL)	0.57 ± 0.03	0.56 ± 0.03	0.57 ± 0.04	0.56 ± 0.02	0.62 ± 0.02	0.61 ± 0.04
Alanine aminotransferase (IU/L)	21 ± 1	21 ± 2	24 ± 2	29 ± 5	23 ± 1	29 ± 2*
Lactate dehydrogenase (IU/L)	576 ± 49	799 ± 64	828 ± 61*	668 ± 56	714 ± 43	594 ± 79
Sorbitol dehydrogenase (IU/L)	5 ± 0	7 ± 0*	9 ± 0**	20 ± 5**	22 ± 2**	25 ± 3**

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error
[b] n=9

TABLE E2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 9-Month Interim Sacrifice in the 2-Year Drinking Water Studies of C.I. Acid Red 114[a]

Analysis	0 ppm	300 ppm
Male[b]		
n	10	10
Hematology		
Hematocrit (%)	43.6 ± 0.5	42.9 ± 0.4
Hemoglobin (g/dL)	16.5 ± 0.1	16.0 ± 0.2*
Erythrocytes ($10^6/\mu L$)	8.67 ± 0.11	8.64 ± 0.08
Mean cell volume (μ^3)	50.2 ± 0.2	49.6 ± 0.3
Mean cell hemoglobin (pg)	19.1 ± 0.2	18.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)	37.9 ± 0.3	37.3 ± 0.4
Leukocytes ($10^3/\mu L$)	6.91 ± 0.23	6.90 ± 0.29
Segmented neutrophils (%)	30.90 ± 2.39	34.70 ± 2.99
Lymphocytes (%)	66.30 ± 2.53	63.20 ± 3.09
Monocytes (%)	1.20 ± 0.33	0.70 ± 0.26
Eosinophils (%)	1.60 ± 0.43	1.30 ± 0.26
Nucleated erythrocytes/leukocytes x 10^2	0.30 ± 0.21	0.10 ± 0.10
n	10	10
Clinical chemistry		
Urea nitrogen (mg/dL)	19.20 ± 0.85	17.90 ± 0.67
Creatinine (mg/dL)	0.69 ± 0.04	0.59 ± 0.02*
Serum glucose (mg/dL)	145 ± 6	146 ± 6
Alanine aminotransferase (IU/L)	64 ± 8	46 ± 3*
Lactate dehydrogenase (IU/L)	791 ± 61	678 ± 77
Sorbitol dehydrogenase (IU/L)	14 ± 3	14 ± 2
T_3 (ng/dL)	66 ± 5	62 ± 3
T_4 (μg/dL)	3 ± 0	3 ± 0
Serum osmolality (mOs/kg)	311 ± 3	307 ± 2
Osmolality ratio	6 ± 1	10 ± 1**
Thyroid-stimulating hormone (ng/mL)	478 ± 44	457 ± 36
n	10	10
Urinalysis		
Urine osmolality (mOsm/kg)	1,766 ± 246	2,954 ± 294**
Creatinine excretion (mg/16 h)	7.44 ± 0.42	7.63 ± 0.55
Urine creatinine (mg/dL)	209.7 ± 23.6	325.0 ± 30.0**
Urine volume (mL/16 h)	4 ± 1	3 ± 0*
Specific gravity	1.048 ± 0.004	1.059 ± 0.001*
Urine pH	6.60 ± 0.10	6.60 ± 0.10

TABLE E2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 9-Month Interim Sacrifice in the 2-Year Drinking Water Studies of C.I. Acid Red 114 (continued)

Analysis	0 ppm	600 ppm
Female[b]		
n	10	10
Hematology		
Hematocrit (%)	44.7 ± 0.6	41.4 ± 1.0*
Hemoglobin (g/dL)	16.1 ± 0.1	15.4 ± 0.2*
Erythrocytes ($10^6/\mu L$)	8.16 ± 0.10	7.56 ± 0.16**
Mean cell volume (μ^3)	54.7 ± 0.2	54.6 ± 0.3
Mean cell hemoglobin (pg)	19.7 ± 0.2	20.4 ± 0.4
Mean cell hemoglobin concentration (g/dL)	36.1 ± 0.4	37.4 ± 0.8
Leukocytes ($10^3/\mu L$)	4.70 ± 0.24	5.65 ± 0.42*
Segmented neutrophils (%)	31.30 ± 3.02	31.40 ± 4.03
Lymphocytes (%)	66.90 ± 3.08	67.00 ± 3.94
Monocytes (%)	0.40 ± 0.16	0.20 ± 0.13
Eosinophils (%)	1.20 ± 0.20	1.40 ± 0.40
Nucleated erythrocytes/leukocytes x 10^2	1.40 ± 0.81	0.00 ± 0.00*
n	10	10
Clinical chemistry		
Urea nitrogen (mg/dL)	15.60 ± 0.52	14.40 ± 0.67
Creatinine (mg/dL)	0.54 ± 0.02	0.51 ± 0.03
Serum glucose (mg/dL)	141 ± 5	148 ± 6
Alanine aminotransferase (IU/L)	26 ± 2	30 ± 4
Lactate dehydrogenase (IU/L)	358 ± 46	520 ± 38*
Sorbitol dehydrogenase (IU/L)	7 ± 1	18 ± 4**
T_3 (ng/dL)	112 ± 5	95 ± 6*[d]
T_4 (μg/dL)	3 ± 0	2 ± 0**[d]
Serum osmolality (mOs/kg)	312 ± 3	312 ± 4
Osmolality ratio	5 ± 1[c]	8 ± 0**[d]
Thyroid-stimulating hormone (ng/mL)	332 ± 29	303 ± 18[d]
n	7	10
Urinalysis		
Urine osmolality (mOsm/kg)	1,673 ± 205	2,445 ± 140**[d]
Creatinine excretion (mg/16 h)	3.43 ± 0.35	2.05 ± 0.51[f]
Urine creatinine (mg/dL)	168.7 ± 20.6	264.4 ± 13.3**[f]
Urine volume (mL/16 h)	2 ± 0[e]	1 ± 0
Specific gravity	1.045 ± 0.004	1.059 ± 0.001**
Urine pH	6.57 ± 0.13	6.35 ± 0.08

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. T_3=Triiodothyronine; T_4=Thyroxine.
[b] No measurements taken for males at 600 ppm or for females at 300 ppm.
[c] n=7
[d] n=9
[e] n=10
[f] n=8

TABLE E3
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 15-Month Interim Sacrifice in the 2-Year Drinking Water Studies of C.I. Acid Red 114[a]

Analysis	0 ppm	70 ppm	150 ppm	300 ppm
Male[b]				
n	10	10	10	10
Hematology				
Hematocrit (%)	46.1 ± 0.7	42.8 ± 1.7	45.1 ± 0.7	39.5 ± 1.2**
Hemoglobin (g/dL)	16.4 ± 0.2	14.9 ± 0.6*	15.7 ± 0.2*	15.0 ± 0.5**
Erythrocytes ($10^6/\mu L$)	9.04 ± 0.14	8.33 ± 0.36	8.87 ± 0.15	7.86 ± 0.24**
Mean cell volume (μ^3)	51.0 ± 0.2	51.5 ± 0.6	50.8 ± 0.2	50.2 ± 0.1**
Mean cell hemoglobin (pg)	18.2 ± 0.2	17.9 ± 0.2	17.8 ± 0.2	19.1 ± 0.3
Mean cell hemoglobin concentration (g/dL)	35.7 ± 0.3	34.7 ± 0.3	35.0 ± 0.3	38.0 ± 0.6*
Leukocytes (μL)	5.37 ± 0.28	5.38 ± 0.40	5.19 ± 0.35	6.41 ± 0.40
Segmented neutrophils (%)	46.90 ± 3.28	45.50 ± 3.28	51.50 ± 1.92	47.50 ± 5.40
Lymphocytes (%)	51.20 ± 3.45	52.50 ± 3.17	46.80 ± 1.84	50.60 ± 5.11
Monocytes (%)	0.50 ± 0.22	0.90 ± 0.18	0.70 ± 0.21	0.80 ± 0.29
Eosinophils (%)	1.30 ± 0.37	1.10 ± 0.31	1.00 ± 0.26	1.10 ± 0.35
Nucleated erythrocytes/leukocytes x 10^2	0.30 ± 0.30	0.80 ± 0.55	0.40 ± 0.27	0.10 ± 0.10
n	10	10	10	10
Clinical chemistry				
Urea nitrogen (mg/dL)	16.60 ± 0.73	16.00 ± 0.30	15.70 ± 0.47	16.20 ± 0.55
Creatinine (mg/dL)	0.69 ± 0.04	0.59 ± 0.01	0.59 ± 0.03	0.61 ± 0.01
Serum glucose (mg/dL)	165 ± 7	151 ± 3	144 ± 6	152 ± 3
Alanine aminotransferase (IU/L)	149 ± 41	129 ± 30	90 ± 15	91 ± 16
Lactate dehydrogenase (IU/L)	795 ± 98	708 ± 82	646 ± 46	526 ± 60*
Sorbitol dehydrogenase (IU/L)	29 ± 5[c]	37 ± 9	31 ± 6	35 ± 7
T_3 (ng/dL)	87 ± 4	92 ± 3[c]	88 ± 4	96 ± 3
T_4 ($\mu g/dL$)	4 ± 0	4 ± 0[c]	4 ± 0[c]	4 ± 0
Serum osmolality (mOs/kg)	312 ± 2	310 ± 3	309 ± 3	310 ± 3
Osmolality ratio	3 ± 0	4 ± 0	5 ± 1	6 ± 1**
Thyroid-stimulating hormone (ng/mL)	255 ± 16	294 ± 45[c]	234.1 ± 20.0	292.5 ± 15.4
n	10	10	10	10
Urinalysis				
Urine osmolality (mOsm/kg)	1,085 ± 97	1,218 ± 117	1,457 ± 195	1,858 ± 206**
Creatinine excretion (mg/16 h)	7.99 ± 0.45	8.39 ± 0.48	8.41 ± 0.43	8.36 ± 0.37[c]
Urine creatinine (mg/dL)	136.4 ± 10.9	160.2 ± 15.9	184.3 ± 26.6	254.0 ± 27.5**
Urine volume (mL/16 h)	6 ± 1	6 ± 1	5 ± 1	4 ± 1**
Specific gravity	1.033 ± 0.003	1.037 ± 0.003	1.043 ± 0.005	1.049 ± 0.004**
Urine pH	6.95 ± 0.05	6.80 ± 0.11	6.65 ± 0.11*	6.35 ± 0.08**

TABLE E3
Hematology, Clinical Chemistry, and Urinalysis Data for Rats at the 15-Month Interim Sacrifice in the 2-Year Drinking Water Studies of C.I. Acid Red 114 (continued)

Analysis	0 ppm	150 ppm	300 ppm[c]	600 ppm
Female[b]				
n	10	10	10	10
Hematology				
Hematocrit (%)	47.2 ± 0.9	47.2 ± 0.6	42.8 ± 1.2*	37.5 ± 1.4**
Hemoglobin (g/dL)	16.2 ± 0.2	15.5 ± 0.3	15.1 ± 0.4**	14.1 ± 0.4**
Erythrocytes ($10^6/\mu L$)	8.60 ± 0.18	8.65 ± 0.08	7.96 ± 0.24	6.99 ± 0.28**
Mean cell volume (μ^3)	54.8 ± 0.3	54.5 ± 0.3	53.7 ± 0.5*	53.6 ± 0.7*
Mean cell hemoglobin (pg)	18.9 ± 0.3	18.0 ± 0.2	19.0 ± 0.4	20.4 ± 0.5*
Mean cell hemoglobin concentration (g/dL)	34.4 ± 0.4	32.9 ± 0.2	35.3 ± 0.7	38.0 ± 1.0*
Leukocytes (μL)	3.32 ± 0.22	3.01 ± 0.10	4.02 ± 0.29	5.36 ± 0.46**
Segmented neutrophils (%)	37.10 ± 2.67	40.00 ± 3.28	48.44 ± 3.53*	54.90 ± 2.83**
Lymphocytes (%)	61.00 ± 2.63	58.40 ± 3.58	50.56 ± 3.35	44.10 ± 2.72**
Monocytes (%)	1.10 ± 0.35	0.30 ± 0.15	0.22 ± 0.15	0.40 ± 0.16
Eosinophils (%)	0.80 ± 0.36	1.00 ± 0.45	0.78 ± 0.22	0.50 ± 0.31
Nucleated erythrocytes/leukocytes x 10^2	0.30 ± 0.30	0.50 ± 0.31	0.89 ± 0.42	0.10 ± 0.10
n	9	10	10	10
Clinical chemistry				
Urea nitrogen (mg/dL)	18.22 ± 0.64	16.60 ± 0.31	15.78 ± 0.46*	21.50 ± 1.80
Creatinine (mg/dL)	0.67 ± 0.04	0.58 ± 0.03	0.64 ± 0.03	0.69 ± 0.02
Serum glucose (mg/dL)	138 ± 4	153 ± 3**	152 ± 5*	176 ± 11**
Alanine aminotransferase (IU/L)	36 ± 3	40 ± 4	44 ± 8	72 ± 14**
Lactate dehydrogenase (IU/L)	425 ± 22	365 ± 46	445 ± 39	588 ± 74
Sorbitol dehydrogenase (IU/L)	11 ± 1	20 ± 4**	27 ± 6**	39 ± 5**
T_3 (ng/dL)	96 ± 3[d]	104 ± 4	109 ± 4*	120 ± 7**
T_4 (μg/dL)	3 ± 0[d]	4 ± 0	3 ± 0	3 ± 0[e]
Serum osmolality (mOs/kg)	306 ± 2	311 ± 2	308 ± 2	315 ± 4
Osmolality ratio	3 ± 0	3 ± 0	3 ± 0	3 ± 1
Thyroid-stimulating hormone (ng/mL)	204 ± 23[d]	217 ± 30	270 ± 41	469 ± 87**
n	10	10	10	10
Urinalysis				
Urine osmolality (mOsm/kg)	1,063 ± 140	799 ± 95	1,023 ± 100	1,078 ± 184
Creatinine excretion (mg/16 h)	3.79 ± 0.25	4.76 ± 0.22*	4.37 ± 0.25	3.52 ± 0.39
Urine creatinine (mg/dL)	143.4 ± 26.6	84.8 ± 9.6	106.4 ± 7.5	114.6 ± 19.5
Urine volume (mL/16 h)	4 ± 1	6 ± 1*	4 ± 0	4 ± 1
Specific gravity	1.031 ± 0.004	1.027 ± 0.003	1.034 ± 0.002	1.042 ± 0.005
Urine pH	6.85 ± 0.08	6.90 ± 0.07	6.72 ± 0.09	6.55 ± 0.09*

* Significantly different ($P \le 0.05$) from the control group by Dunn's or Shirley's test
** $P \le 0.01$
[a] Mean ± standard error. T_3=Triiodothyronine; T_4=Thyroxine.
[b] No measurements taken for males at 600 ppm or for females at 70 ppm.
[c] n=9
[d] n=8
[e] n=5

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

C.I. Acid Red 114 was obtained in a single lot (lot number A101681) from the Atlantic Chemical Company by the analytical chemistry laboratory, Midwest Research Institute (MRI), Kansas City, MO. Lot A101681 was desalted by dialysis in two batches and assigned lot numbers M113081 and M032582, by MRI. The resultant chloride content, expressed as sodium chloride, was reduced from 14.9% to 0.91% for lot M113081, and 0.35% for lot M032582. Reports on purity, stability, and identity analyses performed by MRI in support of the studies are on file at the National Institute of Environmental Health Sciences.

The study dye, a red powder, was identified as C.I. Acid Red 114 by infrared, ultraviolet/visible, and Fourier transform nuclear magnetic resonance spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra of C.I. Acid Red 114 (Figures F1 and F2) *(Sadtler Standard Spectra)*. However, both lots were found to consist of a complex mixture of inorganic and organic impurities. Thus, elemental analysis alone could not be used to confirm the identity or relative purity of the major component.

The purity of the two desalted lots was determined by elemental analysis, Karl Fischer water analysis, azo group titration, potentiometric titration, and chromatographic analyses. The determination of the azo groups was performed by reducing the azo group first with a solution of acetic/hydrochloric acid containing titanium (III) chloride, and then back titrating excess titanium (III) with standardized ferric ammonium sulfate. Normal phase thin-layer chromatography (TLC) was performed on silica gel plates with two solvent systems: 1) 2-butanone:toluene:diethylamine:pyridine:water (26:11:21:21:21) and 2) diethylamine:water (85:15). Visualization was accomplished with visible light and short (254 nm) and long (366 nm) wavelength ultraviolet light. To minimize the formation of the hydrazoquinone tautomer, HPLC was performed with a Supelcosil LC-18-DB column in a mixture of two solvents: A) aqueous pH 7.41 buffer (Fisher pH 7.41 dry buffer salt, prepared according to packet directions):water:methanol (1:9:1, v/v/v), and B) same as solvents as in A, but the ratios of solvent were 0.01:0.9:9, (v/v/v). Both systems used a flow rate of 1 mL/min. Ultraviolet detection was at 254 nm, and visible detection was at 546 nm.

Even though elemental analysis could not be used to confirm the identity or relative purity of the major component because of the complex mixture of impurities, it did indicate the presence of sodium chloride at 0.35% for lot M032582 and 0.9% for lot M113081. Karl Fischer analysis indicated the presence of 1.16% water for lot M032582, and 4.2% water for lot M113081. Titration of the azo groups indicated a purity of 86.5% for lot M032582, and 83% for lot M113081. These purity estimates are based on the presence of two azo groups per molecule and are probably enhanced by the presence of titrable impurities. Thus the purity may be lower than these estimates. Normal phase TLC using solvent system 1 indicated one major component, two minor impurities, six trace impurities, and two slight trace spots. Solvent system 2 indicated one major component, two minor impurities, six trace and three slight trace impurities. Both lots of study dye were analyzed concomitantly by HPLC and found to have similar impurity peak patterns consisting of one major peak and 15 minor impurities. At 254 nm, five of these impurities had relative peak areas greater than 1.0% but less than 3% of the total peak area with overall impurity peak areas of 13.8% for lot M032582, and 13.6% for lot M113081. At 546 nm, four impurities had relative peak areas greater than 1.0% with total peak areas of 13.8% for lot M032582, and 13.7% for lot M113081. A major peak comparison indicated the relative purity of lot M113081 was 92.1% when compared to lot M032582. The overall purity of lot M032582 was estimated at approximately 85% compared to approximately 82% for lot M113081.

Both lots of C.I. Acid Red 114 were analyzed for the presence of benzidine and 3,3′-dimethylbenzidine using an HPLC procedure. A Waters μBondapak C_{18} (300 x 3.9 nm ID) column and a fluorescence

detector were employed. The excitation wavelength was 285 nm for benzidine, 275 nm for 3,3′-dimethylbenzidine, and the emission was monitored using a 389 nm cutoff filter. The mobile phases were 82% 0.01 M phosphate buffer (pH 7): 18% acetonitrile for benzidine and 66% 0.01 M phosphate buffer (pH 7.4): 34% methanol for 3,3′-dimethylbenzidine. A flow rate of 1.5 mL/min was used. Benzidine could not be detected in either lot at levels greater than 1 ppm; whereas, 3,3′-dimethylbenzidine was found in both lots at concentrations of approximately 5 ppm.

Stability studies were performed by HPLC with acetanilide as an internal standard and with ultraviolet detection at 254 nm. When stored protected from light, the study material was stable as a bulk chemical for at least 2 weeks at temperatures up to 60° C. During all studies the study laboratory (Hazleton Laboratories America) monitored the stability of the bulk chemical by HPLC and ultraviolet/visible spectrophotometry, and no loss of purity was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The drinking water dose formulations were prepared using tap water (Fairfax County Water Authorities, VA) as the vehicle for 13-day studies, and distilled water (Polar Water Co., Beltsville, MD) as the vehicle for the 13-week and 2-year studies. The appropriate amount of C.I. Acid Red 114 for each dose level was weighed and added to the appropriate volume of vehicle. At room temperature, the maximum solubility of the study material was 4.6 mg/mL (4,600 ppm). Since dose levels above 4,600 ppm were planned for the 13-day study, tests were conducted to improve solubility. Adjusting pH, mixing with solvents (ethanol and acetone), and using buffer salts (citrates or acetates) did not improve solubility. However, increasing the temperature of the water vehicle to 75° C increased solubility to 30 mg/mL (30,000 ppm). This supersaturated solution was physically stable for approximately 36 hours. Mixing the dye at 75° C had no adverse effect on the chemical, as determined from absorbance spectra, nor did reheating the dose solution to the same temperature. Thus, for the 13-day studies, the tap water vehicle was heated to 75° C before mixing with the dye. Sampling errors were evident during the analyses of the 13-day dose solutions (Table F2), and these were considered to be due in part to gradual settling out of the dye during storage.

Stability studies were conducted at the analytical laboratory. The concentration of C.I. Acid Red 114 was determined by HPLC with a μBondapak C_{18} column and a mobile phase of 30% water with 0.1% triethanolamine, pH 7.2, and 70% methanol with 0.1% triethanolamine, pH 7.2. The pH was adjusted to 7.2 with phosphoric acid. The flow rate was 1.0 mL/min and visible detection was at 512 or 546 nm.

C.I. Acid Red 114 at 375 ppm was found to be stable in water for up to 21 days when stored protected from light in sealed amber containers at room temperature. Solutions stored at 5° C showed measurable loss due to precipitation. Storage under simulated animal cage conditions (open to air and light) for 72 hours had no measurable affect on chemical stability.

Periodic analyses of the dose formulations of C.I. Acid Red 114 were conducted at the study laboratory and at the analytical laboratory using ultraviolet spectroscopy. For the 13-day studies, dose formulations were analyzed prior to study initiation and at study termination (Table F2). For the 13-week studies, dose formulations were analyzed prior to study initiation, at the midpoint of the study (collected from both the dose preparation area and from the animal room), and at the end of the study (Table F3). During the 2-year studies, one of every eight sets of the dose formulations were analyzed by ultraviolet spectroscopy. Animal room dose solutions were analyzed approximately every three months, and after the completion of each dosing interval. Results of the dose formulation analyses for the 2-year studies are presented in Table F4. All formulations were within ± 10% of the target concentration. Results of periodic referee analysis performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table F5).

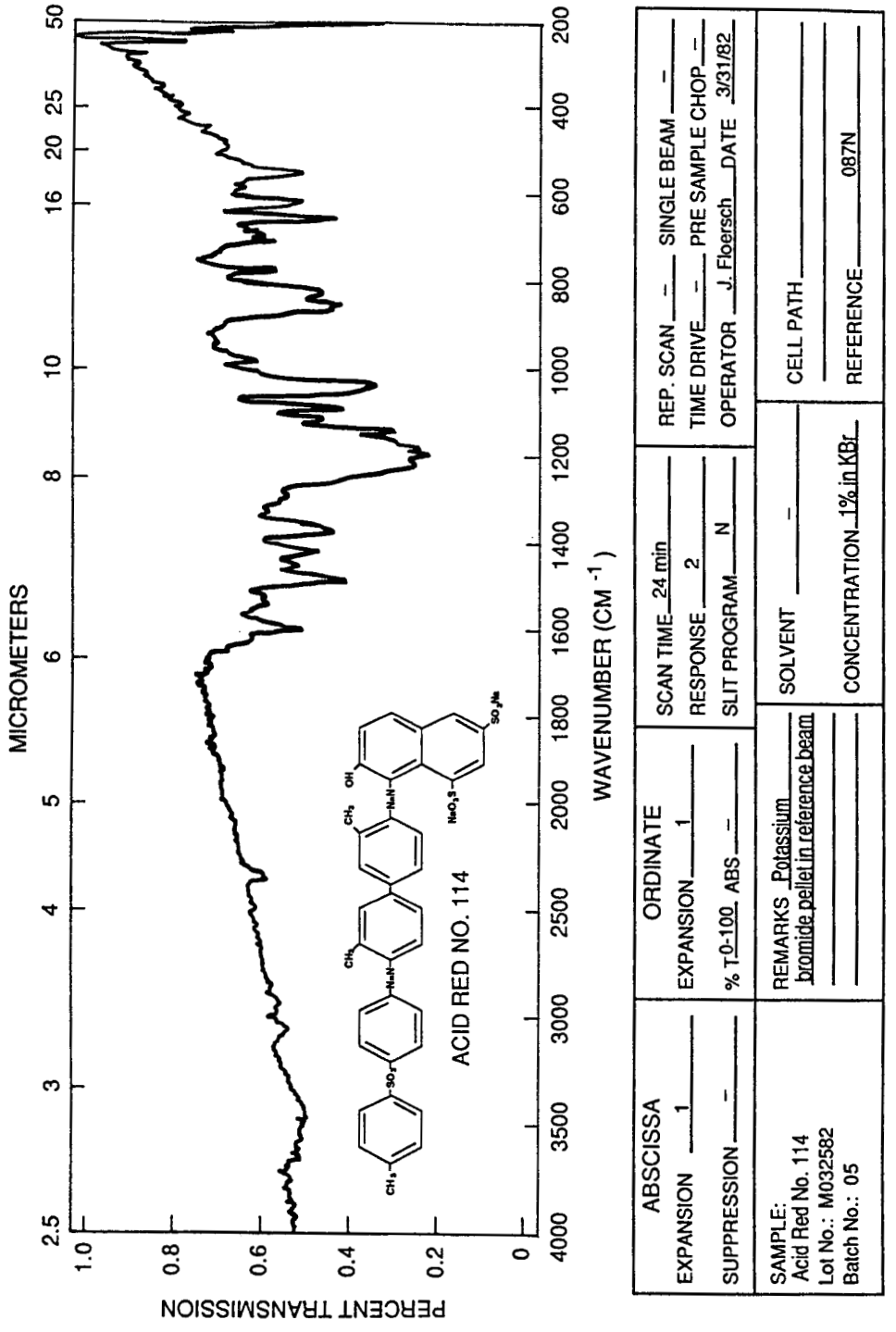

Figure F1
Infrared Absorption Spectrum of C.I. Acid Red 114

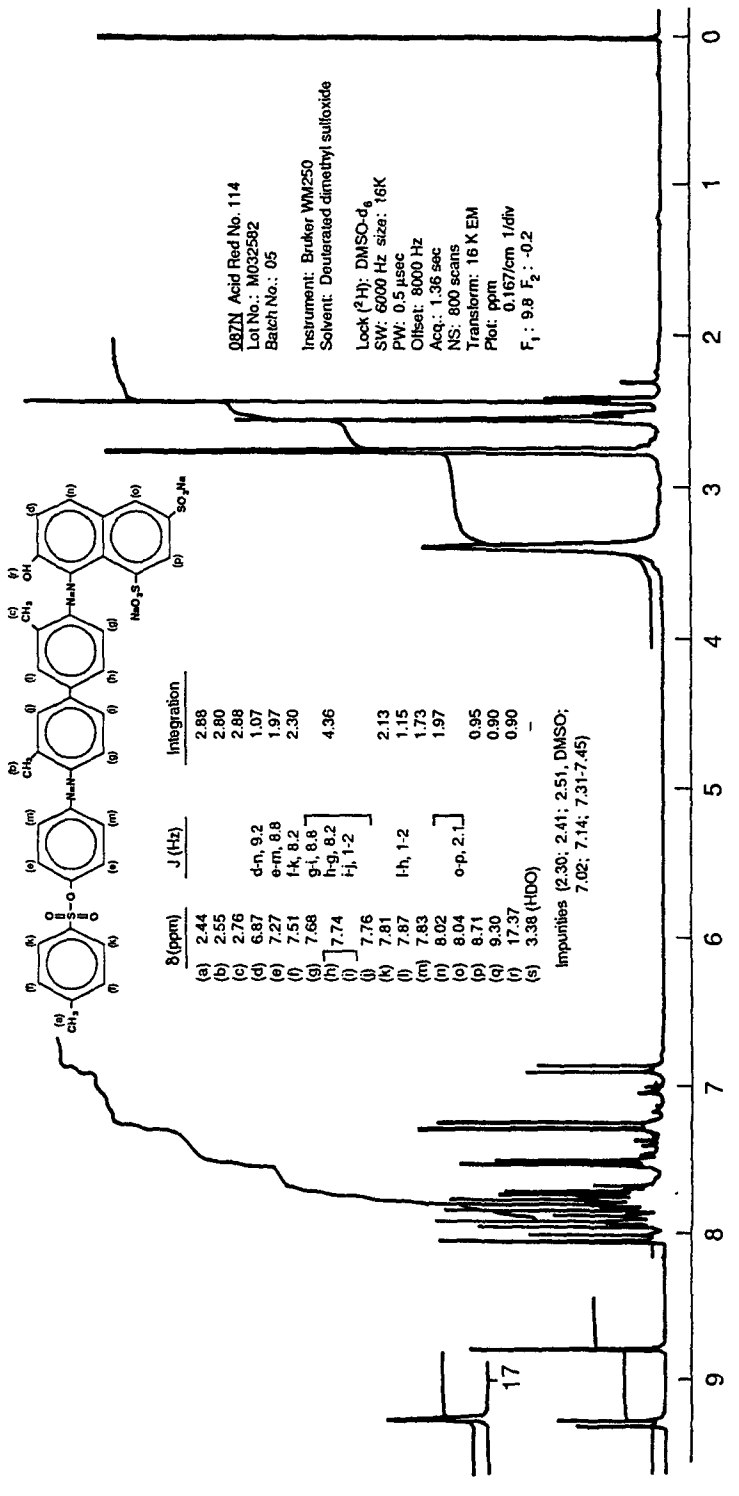

Figure F2
Nuclear Magnetic Resonance Spectrum of C.I. Acid Red 114

TABLE F1
Preparation and Storage of Dose Formulations in the Drinking Water Studies of C.I. Acid Red 114

13-Day Studies	13-Week Studies	2-Year Studies
Preparation		
Weighed amount of C.I. Acid Red 114 was placed in a carboy. The appropriate amount of tap water was added, and the solution was mixed continuously with an electric stirrer until the chemical dissolved. For doses above 4,500 ppm, the weighed amount of C.I. Acid Red 114 was added to tap water heated to 75° C, stirred without heat, and allowed to cool to room temperature.	Same as 13-day studies, except vehicle was distilled water (Polar Water Co., Beltsville, MD).	Weighed amount of C.I. Acid Red 114 was placed in a carboy. The appropriate amount of distilled water was added, and the solution was mixed continuously with an electric stirrer until the chemical dissolved.
Chemical Lot Number		
M113081	M032582	M032582
Maximum Storage Time		
3-4 days	3-4 days	1 week
Storage Conditions		
In the dark at room temperature	Same as 13-day studies	Same as 13-day studies

TABLE F2
Results of Analysis of Dose Formulations in the 13-Day Drinking Water Studies of C.I. Acid Red 114

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
30 March 1982	31 March 1982	10,000	7,624	-24
		20,000	14,000	-30
		30,000	20,110	-33
2 April 1982[b]	2 April 1982	10,000	11,320	+13
		20,000	21,618	+8
		30,000	33,950	+13
9 April 1982	13 April 1982	10,000	2,833	-72
		20,000	9,952	-50
		30,000	16,683	-44

[a] Results of duplicate analyses
[b] Second mix

TABLE F3
Results of Analysis of Dose Formulations in the 13-Week Drinking Water Studies of C.I. Acid Red 114

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
27 July 1982	27 July 1982	600	650	+8
		1,200	1,280	+7
		2,500	2,620	+5
		5,000	5,300	+6
		10,000	10,170	+2
29 July 1982[b]	30 July 1982	600	640	+7
		1,200	1,260	+5
		2,500	2,660	+6
		5,000	5,200	+4
		10,000	10,400	+4
9 September 1982	10 September 1982	600	640	+7
		1,200	1,260	+5
		2,500	2,570	+3
		5,000	5,350	+7
		10,000	10,740	+7
9 September 1982[b]	10 September 1982	600	650	+8
		1,200	1,280	+7
		2,500	2,620	+5
		5,000	5,270	+5
		10,000	10,670	+7
28 October 1982	28 October 1982	600	640	+7
		1,200	1,270	+6
		2,500	2,650	+6
		5,000	5,320	+6
		10,000	10,520	+5

[a] Results of duplicate analyses.
[b] Animal room samples

TABLE F4
Results of Analysis of Dose Formulations in the 2-Year Drinking Water Studies of C.I. Acid Red 114

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
3 June 1983	8 June 1983	70	70	0
		150	150	0
		300	290	-3
		300	280	-7
		600	580	-3
3 June 1983[b]	15 June 1983	70	70	0
		150	150	0
		300	290	-3
		300	280	-7
		600	580	-3
28 June 1983	1 July 1983	70	70	0
		150	150	0
		300	300	0
		300	310	+3
		600	610	+2
26 July 1983	27 July 1983	70	66	-6
		150	156	+4
		300	308	+3
		300	310	+3
		600	604	+1
26 July 1983[b]	9 August 1983	70	64	-8
		150	153	+2
		300	310	+3
		300	312	+4
		600	606	+1
23 August 1983	24 August 1983	70	74	+6
		150	158	+5
		300	316	+5
		300	308	+3
		600	615	+3
20 September 1983	23 September 1983	70	71	+1
		150	155	+3
		300	317	+6
		300	315	+5
		600	613	+2
18 October 1983	20 October 1983	70	70	0
		150	153	+2
		300	295	-2
		300	307	+2
		600	567	-5

TABLE F4
Results of Analysis of Dose Formulations in the 2-Year Drinking Water Studies of C.I. Acid Red 114
(continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
18 October 1983[b]	1 November 1983	70	74	+6
		150	156	+4
		300	301	0
		300	305	+2
		600	567	-5
15 November 1983	17 November 1983	70	68	-3
		150	151	0
		300	296	-1
		300	299	0
		600	582	-3
13 December 1983	16 December 1983	70	71	+1
		150	153	+2
		300	302	+1
		300	303	+1
		600	580	-3
10 January 1984	13 January 1984	70	74	+6
		150	151	+1
		300	295	-2
		300	305	+2
		600	579	-3
10 January 1984[b]	27 January 1984	70	65	-7
		150	136	-9
		300	284	-5
		300	286	-5
		600	553	-8
7 February 1984	10 February 1984	70	76	+8
		150	154	+3
		300	301	0
		300	303	+1
		600	602	0
6 March 1984	9 March 1984	70	68	-3
		150	147	-2
		300	292	-3
		300	297	-1
		600	562	-6
3 April 1984	6 April 1984	70	72	+3
		150	154	+3
		300	313	+4
		300	328	+9
		600	612	+2

TABLE F4
Results of Analysis of Dose Formulations in the 2-Year Drinking Water Studies of C.I. Acid Red 114
(continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
3 April 1984[b]	13 April 1984	70	70	0
		150	152	+1
		300	306	+2
		300	323	+8
		600	598	0
1 May 1984	3 May 1984	70	69	-1
		150	151	+1
		300	304	+1
		300	304	+1
		600	601	0
29 May 1984	1 June 1984	70	70	0
		150	151	+1
		300	310	+3
		300	307	+2
		600	612	+2
26 June 1984	28 June 1984	70	67	-4
		150	150	0
		300	305	+2
		300	309	+3
		600	586	-2
26 June 1984[b]	10 July 1984	70	72	+3
		150	156	+4
		300	313	+4
		300	318	+6
		600	625	+4
24 July 1984	27 July 1984	70	73	+4
		150	157	+5
		300	311	+4
		300	311	+4
		600	593	-1
21 August 1984	23 August 1984	70	75	+7
		150	163	+9
		300	317	+6
		300	314	+5
		600	612	+2
18 September 1984	21 September 1984	70	73	+4
		150	157	+5
		300	313	+4
		300	302	+1
		600	592	-1

TABLE F4
Results of Analysis of Dose Formulations in the 2-Year Drinking Water Studies of C.I. Acid Red 114
(continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
17 October 1984	22 October 1984	70	73	+4
		150	155	+3
		300	307	+2
		300	311	+2
		600	577	-4
17 October 1984[b]	27 October 1984	70	75	+7
		150	152	+1
		300	305	+2
		300	307	+2
		600	580	-3
13 November 1984	15 November 1984	70	66	-6
		150	149	-1
		300	301	0
		300	298	-1
		600	568	-5
11 December 1984	13 December 1984	70	70	0
		150	151	+1
		300	305	+2
		300	309	+3
		600	609	+2
11 December 1984[b]	21 December 1984	70	71	+1
		150	154	+3
		300	310	+3
		300	310	+3
		600	574	-4
8 January 1985	11 January 1985	70	65	-7
		150	140	-7
		300	281	-6
		300	280	-7
		600	556	-7
5 February 1985	8 February 1985	70	70	0
		150	153	+2
		300	301	0
		300	301	0

TABLE F4
Results of Analysis of Dose Formulations in the 2-Year Drinking Water Studies of C.I. Acid Red 114
(continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
5 March 1985	8 March 1985	70	65	-7
		150	146	-3
		300	290	-3
		300	297	-1
5 March 1985[b]	16 March 1985	70	75	+7
		150	153	+2
		300	302	+1
		300	308	+3
2 April 1985	5 April 1985	70	76	+8
		150	163	+9
		300	309	+3
		300	305	+2
7 May 1985	9 May 1985	70	74	+6
		150	161	+7
		300	312	+4
		300	320	+7
21 May 1985	23 May 1985	70	71	+1
		150	154	+3
		300	313	+4
		300	307	+2
21 May 1985[b]	3 June 1985	70	72	+3
		150	150	0
		300	303	+1
		300	302	+1

[a] Results of duplicate analyses.
[b] Animal room samples

TABLE F5
Results of Referee Analysis of Dose Formulations in the 2-Year Drinking Water Studies of C.I. Acid Red 114

Date Mixed	Target Concentration	Determined Concentration (ppm)	
		Study Laboratory[a]	Referee Laboratory[b]
3 June 1983	300	290	285
		280	277
20 December 1983	600	–[c]	558
3 April 1984	70	72	69
17 October 1984	150	155	146
7 May 1985	300	312	289

[a] Results of duplicate analyses
[b] Results of triplicate analyses
[c] Data not available

APPENDIX G
WATER AND COMPOUND CONSUMPTION IN THE 2-YEAR STUDIES

TABLE G1
Water and Compound Consumption by Male Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

	0 ppm		70 ppm			150 ppm			300 ppm		
Week	Water (g/day)[a]	Body Wt. (g)	Water (g/day)[a]	Body Wt. (g)	Dose/ Day[b]	Water (g/day)[a]	Body Wt. (g)	Dose/ Day[b]	Water (g/day)[a]	Body Wt. (g)	Dose/ Day[b]
4	24.4	227	22.6	228	7.0	23.7	230	15.4	22.5	227	29.8
5	24.0	249	25.0	255	6.9	23.9	251	14.3	24.1	253	28.6
8	24.9	300	21.5	302	5.0	23.8	305	11.7	23.6	297	23.8
9	24.2	314	22.6	314	5.0	22.5	319	10.6	22.3	315	21.3
12	22.1	346	21.8	349	4.4	19.2	354	8.1	18.5	348	16.0
13	22.1	352	21.7	347	4.4	22.3	358	9.3	21.7	352	18.5
17	25.3	370	26.9	377	5.0	24.9	382	9.8	27.1	378	21.5
21	23.5	396	31.7	391	5.7	27.9	392	10.7	28.9	387	22.4
25	22.8	418	23.7	415	4.0	24.3	422	8.6	22.7	421	16.1
29	21.9	423	22.4	423	3.7	22.2	432	7.7	20.3	428	14.3
33	23.4	436	23.2	438	3.7	23.2	437	8.0	20.6	435	14.2
37	22.0	443	19.8	442	3.1	20.5	449	6.9	19.9	443	13.5
41	26.0	449	22.5	455	3.5	22.9	452	7.6	21.7	450	14.5
45	23.1	457	21.7	455	3.3	22.8	457	7.5	23.7	455	15.7
49	21.0	462	19.2	459	2.9	20.4	463	6.6	19.4	458	12.7
53	20.4	460	18.4	463	2.8	18.6	466	6.0	17.7	456	11.6
57	23.0	454	21.9	453	3.4	21.8	453	7.2	21.3	445	14.3
61	20.4	454	19.3	459	2.9	20.7	462	6.7	19.3	451	12.8
65	20.6	459	19.9	460	3.0	20.8	463	6.7	20.7	452	13.7
69	21.6	458	23.7	452	3.7	24.0	455	7.9	22.9	442	15.6
73	18.6	459	17.8	450	2.8	18.1	451	6.0	19.9	435	13.8
77	22.2	457	22.2	452	3.4	22.6	452	7.5	26.8	436	18.4
81	26.5	450	26.6	447	4.2	23.8	445	8.0	27.7	427	19.5
85	21.1	445	23.2	448	3.6	22.8	443	7.7	26.4	411	19.2
89	21.9	455	21.4	444	3.4	23.0	437	7.9	27.7	400	20.8
93	23.3	445	21.8	427	3.6	22.5	421	8.0	43.4	388	33.6
97	27.5	439	24.8	422	4.1	26.7	407	9.8	37.9	359	31.7
101	22.5	434	25.6	417	4.3	26.7	410	9.8	35.7	352	30.4
Mean for weeks											
1-13	23.6	298	22.5	299	5.4	22.6	303	11.6	22.1	299	23.0
14-52	23.2	428	23.5	428	3.9	23.2	432	8.1	22.7	428	16.1
53-101	22.3	451	22.0	446	3.5	22.5	444	7.6	26.7	420	19.6

[a] Grams of water consumed per animal per day
[b] Estimated milligrams of C.I. Acid Red 114 consumed per day per kilogram of body weight

TABLE G2
Water and Compound Consumption by Female Rats in the 2-Year Drinking Water Study of C.I. Acid Red 114

Week	0 ppm Water (g/day)[a]	0 ppm Body Wt. (g)	150 ppm Water (g/day)[a]	150 ppm Body Wt. (g)	150 ppm Dose/ Day[b]	300 ppm Water (g/day)[a]	300 ppm Body Wt. (g)	300 ppm Dose/ Day[b]	600 ppm Water (g/day)[a]	600 ppm Body Wt. (g)	600 ppm Dose/ Day[b]
4	20.8	150	22.5	148	22.8	21.8	146	44.8	21.5	145	88.8
5	20.7	159	21.9	162	20.3	18.9	155	36.6	17.7	156	68.2
8	22.4	183	23.6	184	19.3	21.9	176	37.4	20.1	174	69.2
9	20.6	187	23.3	186	18.8	19.4	180	32.3	21.4	181	71.1
12	21.3	201	29.2	204	21.5	21.8	194	33.7	17.3	191	54.6
13	18.4	204	19.6	203	14.5	18.5	196	28.3	16.2	193	50.5
17	27.7	216	30.1	214	21.1	19.7	210	28.2	18.2	208	52.5
21	23.3	219	25.4	220	17.3	28.1	211	39.9	22.6	209	65.0
25	19.8	231	22.6	232	14.6	23.8	224	31.9	17.4	221	47.4
29	20.1	235	18.5	233	11.9	19.1	229	25.0	17.5	223	47.0
33	19.4	242	18.1	244	11.1	28.1	231	36.5	18.8	228	49.4
37	17.4	247	16.6	248	10.0	17.4	239	21.8	15.2	233	39.2
41	16.8	255	18.4	259	10.7	17.1	244	21.1	18.1	237	45.9
45	19.4	263	17.4	269	9.7	18.6	252	22.1	17.1	242	42.5
49	17.0	272	16.3	274	8.9	16.7	260	19.2	16.8	242	41.8
53	17.6	286	15.1	290	7.8	16.6	267	18.6	17.4	249	41.8
57	17.5	290	16.2	293	8.3	16.5	278	17.8	18.6	250	44.5
61	16.7	300	15.5	305	7.6	15.9	287	16.6	24.8	250	59.4
65	18.0	307	17.3	312	8.3	17.3	292	17.8	23.3	254	55.1
69	19.8	320	19.2	315	9.2	18.4	296	18.6	28.0	252	66.7
73	17.0	330	18.3	323	8.5	15.2	300	15.2	30.0	271	66.6
77	26.4	334	23.9	332	10.8	19.6	309	19.0	41.9	258	97.6
81	22.2	344	24.8	335	11.1	21.5	307	21.0	38.1	270	84.5
85	18.0	345	22.1	341	9.7	22.7	306	22.3	42.5	249	102.5
89	20.2	352	21.9	341	9.6	19.9	306	19.5			
93	20.7	352	21.0	346	9.1	25.4	300	25.4			
97	23.0	353	25.3	345	11.0	28.6	288	29.8			
101	20.9	358	24.9	349	10.7	25.1	298	25.3			
Mean for weeks											
1-13	20.7	181	23.4	181	19.5	20.4	174	35.5	19.0	173	67.1
14-52	20.1	242	20.4	244	12.8	21.0	233	27.3	18.0	227	47.8
53-101	19.8	329	20.4	325	9.4	20.2	295	20.5	29.4	256	68.7

[a] Grams of water consumed per animal per day
[b] Estimated milligrams of C.I. Acid Red 114 consumed per day per kilogram of body weight

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

TABLE H1
Ingredients of NIH-07 Rat and Mouse Ration[a]

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

[a] NCI, 1976; NIH, 1978. The lot milled 7 May 1985 was not included because it did not meet National Toxicology Program (NTP) standards.
[b] Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE H2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyroxidine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE H3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.57 ± 0.94	21.0–24.9	23
Crude fat (% by weight)	5.47 ± 0.76	3.3–6.5	23
Crude fiber (% by weight)	3.50 ± 0.26	2.8–3.8	23
Ash (% by weight)	6.64 ± 0.32	6.18–7.31	23
Amino Acids (% of total diet)			
Arginine	1.320 ± 0.072	1.310–1.390	5
Cystine	0.319 ± 0.088	0.218–0.400	5
Glycine	1.146 ± 0.063	1.060–1.210	5
Histidine	0.571 ± 0.026	0.531–0.603	5
Isoleucine	0.914 ± 0.030	0.881–0.944	5
Leucine	1.946 ± 0.056	1.850–1.990	5
Lysine	1.280 ± 0.067	1.200–1.370	5
Methionine	0.436 ± 0.165	0.306–0.699	5
Phenylalanine	0.938 ± 0.158	0.665–1.050	5
Threonine	0.855 ± 0.035	0.824–0.898	5
Tryptophane	0.277 ± 0.221	0.156–0.671	5
Tyrosine	0.618 ± 0.086	0.564–0.769	5
Valine	1.108 ± 0.043	1.050–1.170	5
Essential Fatty Acids (% of total diet)			
Linoleic	2.290 ± 0.313	1.830–2.520	5
Linolenic	0.258 ± 0.040	0.210–0.308	5
Vitamins			
Vitamin A (IU/kg)	11,709 ± 4,846	4,100–24,000	23
Vitamin D (IU/kg)	4,450 ± 1,382	3,000–6,300	4
α–Tocopherol (ppm)	43.58 ± 6.92	31.1–48.0	5
Thiamine (ppm)	19.87 ± 3.29	12.0–27.0	23
Riboflavin (ppm)	7.60 ± 0.85	6.10–8.20	5
Niacin (ppm)	97.80 ± 31.68	65.0–150.0	5
Pantothenic acid (ppm)	30.06 ± 4.31	23.0–34.0	5
Pyridoxine (ppm)	7.68 ± 1.31	5.60–8.80	5
Folic acid (ppm)	2.62 ± 0.89	1.80–3.70	5
Biotin (ppm)	0.254 ± 0.053	0.19–0.32	5
Vitamin B_{12} (ppb)	24.21 ± 12.66	10.6–38.0	5
Choline (ppm)	3,122 ± 416.8	2,400–3,430	5
Minerals			
Calcium (%)	1.24 ± 0.15	0.95–1.54	23
Phosphorus (%)	0.95 ± 0.06	0.87–1.01	23
Potassium (%)	0.900 ± 0.098	0.772–0.971	3
Chloride (%)	0.513 ± 0.114	0.380–0.635	5
Sodium (%)	0.323 ± 0.043	0.258–0.371	5
Magnesium (%)	0.167 ± 0.012	0.151–0.181	5
Sulfur (%)	0.304 ± 0.064	0.268–0.420	5
Iron (ppm)	410.3 ± 94.04	262.0–523.0	5
Manganese (ppm)	90.29 ± 7.15	81.70–99.40	5
Zinc (ppm)	52.78 ± 4.94	46.10–58.20	5
Copper (ppm)	10.72 ± 2.76	8.090–15.39	5
Iodine (ppm)	2.95 ± 1.05	1.52–3.82	4
Chromium (ppm)	1.85 ± 0.25	1.44–2.09	5
Cobalt (ppm)	0.681 ± 0.14	0.490–0.780	4

TABLE H4
Contaminant Levels in NIH-07 Rat and Mouse Ration

	Mean ± Standard Deviation[a]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.58 ± 0.20	0.17–0.94	23
Cadmium (ppm)	<0.10	–	23
Lead (ppm)	0.64 ± 0.26	0.33–1.32	23
Mercury (ppm)	<0.05	–	23
Selenium (ppm)	0.33 ± 0.06	0.21–0.42	23
Aflatoxins (ppb)	<5.0	–	23
Nitrate nitrogen (ppm)[b]	9.95 ± 5.23	0.10–19.0	23
Nitrite nitrogen (ppm)[b]	0.79 ± 1.70	0.10–7.20	23
BHA (ppm)[c]	2.13 ± 0.63	2.00–5.00	23
BHT (ppm)[c]	2.17 ± 1.19	1.00–4.00	23
Aerobic plate count (CFU/g)[d]	58,143 ± 43,380	770–130,000	23
Coliform (MPN/g)[e,f]	41.65 ± 104	3.00–460	23
Coliform (MPN/g)[g]	12.29 ± 15.48	3.00–43	21
E. coli (MPN/g)[h]	3.04 ± 0.21	3.00–4	23
Total nitrosamines (ppb)[i]	7.34 ± 6.34	1.80–30.90	23
N-Nitrosodimethylamine (ppb)[i]	6.23 ± 6.25	0.80–30.00	23
N-Nitrosopyrrolidine (ppb)[i]	1.10 ± 0.52	0.90–3.40	23
Pesticides (ppm)			
α-BHC[j]	<0.01		23
β-BHC	<0.02		23
γ-BHC	<0.01		25
δ-BHC	<0.01		23
Heptachlor	<0.01		23
Aldrin	<0.01		23
Heptachlor epoxide	<0.01		23
DDE	<0.01		23
DDD	<0.01		23
DDT	<0.01		23
HCB	<0.01		23
Mirex	<0.01		23
Methoxychlor	<0.05		23
Dieldrin	<0.01		23
Endrin	<0.01		23
Telodrin	<0.01		23
Chlordane	<0.05		23
Toxaphene	<0.1		23
Estimated PCBs	<0.2		23
Ronnel	<0.01		23
Ethion	<0.02		23
Trithion	<0.05		23
Diazinon	<0.1		23
Methyl parathion	<0.02		23
Ethyl parathion	<0.02		23
Malathion[k]	0.15 ± 0.15	0.05–0.65	23
Endosulfan I	<0.01		23
Endosulfan II	<0.01		23
Endosulfan sulfate	<0.03		23

TABLE H4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Source of contamination: alfalfa, grains, and fish meal.
[c] Source of contamination: soy oil and fish meal
[d] CFU = colony forming unit
[e] MPN = most probable number
[f] Mean, standard deviation, and range include two large values of 460 and 240 MPN obtained in the batches milled 20 September 1983 and 14 September 1984, respectively.
[g] Mean, standard deviation, and range exclude the values given in [f].
[h] One lot milled 17 October 1984 has a value of 4.0 MPN.
[i] All values were corrected for percent recovery.
[j] BHC = hexachlorocyclohexane or benzene hexachloride
[k] Fifteen lots contained more than 0.05 ppm.

APPENDIX I
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from randomly selected rats during both the subchronic and chronic studies. Blood from each animal was collected, allowed to clot, and the serum separated. Serum was diluted with physiologic saline solution on a 1:5 ratio and heated to 56° C for 30 minutes prior to shipping to Microbiological Associates, Bethesda, MD, for determination of viral antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times during the studies at which blood was collected for serological testing are also listed.

Method and Test		Time of Analysis
Complement Fixation:		
	RCV (rat coronavirus) Sendai	Preinitiation and termination of 13-week studies.
ELISA:		
	RCV/SDA (sialodacryoadenitis virus)	Preinitiation, 6, 12, 18, and 24 months of 2-year studies.
	PVM, Sendai, *Mycoplasma arthritidis*	At 18 and 24 months of 2-year studies; termination of 22-month studies.
	Mycoplasma pulmonis	Preinitiation, 18, and 24 months of 2-year studies.
Hemagglutination Inhibition:		
	PVM (pneumonia virus of mice)	Preinitiation and termination 13-week studies; preinitiation, 6, and 12 months 2-year studies.
	Sendai	Preinitiation, 6, and 12 months 2-year studies.
	KRV (Kilham rat virus) H-1 (Toolan's H-1 virus)	Preinitiation and termination 13-week studies; preinitiation, 6, 12, 18, and 24 months 2-year studies.

RESULTS

The serology results for sentinel animals are presented in Table I1.

TABLE I1
Murine Virus Antibody Determinations for Rats in the 2-Year Drinking Water Studies of C.I. Acid Red 114

	Interval	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
13-Week Studies	0	0/10	none
	13 weeks	0/10	none
2-Year Studies	0	0/10	none
	6 months	0/10	none
	12 months	1/10	KRV
	18 months	4/10 1/10	possible *M. arthritidis* KRV
	24 months	1/10 3/10	possible *M. arthritidis* KRV

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TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichloroethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	Telone II® (1,3-Dichloropropene)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl) phosphonium Sulfate & Tetrakis(hydroxymethyl) phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3
336	Penicillin VK
337	Nitrofurazone

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338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	*a*-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3'-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
386	Tetranitromethane
387	Amphetamine Sulfate
389	Sodium Azide
390	3,3'-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
393	Sodium Fluoride
395	Probenecid
399	Titanocene Dichloride

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NIH Publication No. 92-3136
December 1991