NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 397

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

C.I. DIRECT BLUE 15

(CAS NO. 2429-74-5)

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 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.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from the NTP Central Data Management, NIEHS, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709 (919-541-3991).

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF C.I. DIRECT BLUE 15
(DESALTED INDUSTRIAL GRADE)
(CAS NO. 2429-74-5)

IN F344/N RATS

(DRINKING WATER STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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August 1992

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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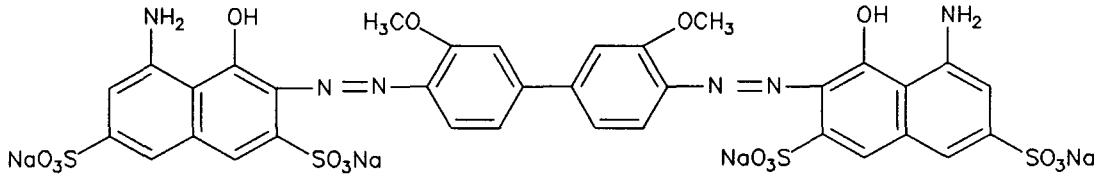
ABSTRACT



C.I. DIRECT BLUE 15

CAS No. 2429-74-5

Chemical Formula: $C_{34}H_{24}N_6O_{16}S_4Na_4$ Molecular Weight: 992.8

Synonyms: Airedale Blue D, Aizen Direct Sky Blue 5BH, Amanil Sky Blue, Atlantic Sky Blue A, Atul Direct Sky Blue, Azine Sky Blue 5B, Belamine Sky Blue A, Benzanil Sky Blue, Benzo Sky Blue S, Benzo Sky Blue A-CF, Cartasol Blue 2GF, Chloramine Sky Blue A, Chloramine Sky Blue 4B, Chrome Leather Pure Blue, C.I. 24400, Cresotine Pure Blue, Diacotton Sky Blue 5B, Diamine Blue 6B, Diamine Sky Blue, Diaphtamine Pure Blue, Diazol Pure Blue 4B, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt, Diphenyl Brilliant Blue, Diphenyl Sky Blue 6B, Direct Blue 10G, Direct Blue HH, Direct Pure Blue, Direct Pure Blue M, Direct Sky Blue (6CI), Direct Sky Blue A, Direct Sky Blue 5B, Enianil Pure Blue AN, Fenamin Sky Blue, Hispamin Sky Blue 3B, Kayafect Blue Y, Kayaku Direct Sky Blue 5B, Mitsui Direct Sky Blue 5B, Naphtamine Blue 10G, Niagara Blue 4B, Niagara Sky Blue, Nippon Direct Sky Blue, Nitto Direct Sky Blue 5B, Paper Blue S, Phenamine Sky Blue A, Pontamine Sky Blue 5BX, Shikiso Direct Sky Blue 5B, Sky Blue 4B, Sky Blue 5B, Tertrodirect Blue F, Vondacel Blue HH

C.I. Direct Blue 15 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. The dye, industrial grade C.I. Direct Blue 15, was chosen for study as a product to which workers are potentially exposed. Because of the high salt content, the dye was desalted prior to use. The purity was determined to be approximately 50%, with high-performance liquid chromatography indicating one major peak and approximately 35 impurities. Toxicology and carcinogenesis studies were conducted by administering the dye, C.I. Direct Blue 15, in drinking water to groups of F344/N rats of each sex for 14 days, 13 weeks, or 22 months. Planned as 24-month studies, the 22-month studies were terminated early because of rapidly declining animal survival, which was due primarily to neoplasia. 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* and Chinese hamster ovary cells.

14-Day Studies

Rats were given C.I. Direct Blue 15 in drinking water at doses of 1,250, 2,500, 5,000, 10,000, or 30,000 ppm. All control and treated rats survived. Body weight gain in high-dose females was less than that in controls. Water consumption declined as the dose increased. Male and female rats receiving 30,000 ppm had slight degeneration and necrosis of individual hepatocytes in the liver, and females also had mild to moderate renal tubule degeneration and thymic lymphoid depletion.

13-Week Studies
C.I. Direct Blue 15 was administered in drinking water at doses of 0, 1,250, 2,500, 5,000, 10,000, or 30,000 ppm to male rats, and at doses of 0, 630, 1,250, 2,500, 5,000, or 10,000 ppm to female rats. Seven of 10 male rats receiving 30,000 ppm died; all rats in the other groups survived until the end of the studies. Mean final body weights of males receiving 10,000 or 30,000 ppm were 92% and 69% of those of controls, and mean final body weights of females receiving 5,000 or 10,000 ppm were 97% and 94% of those of controls. Tissues from treated animals were stained blue. Compound-related lesions were seen in the kidney and liver of male rats given 30,000 ppm and in the kidney of males and females given 10,000 ppm. The renal lesions included necrosis, degeneration, pigmentation and regeneration of the tubule epithelium, and tubule mineralization. Liver lesions included centrilobular hepatocellular degeneration, fatty metamorphosis, and individual cell necrosis with slight periportal hepatocellular hypertrophy. Lymphoid depletion in the thymus was also seen in the high-dose males. Based on the results of the 14-day and 13-week studies, the high dose chosen for the 22-month studies was 2,500 ppm.

22-Month Studies
At study initiation, 70 rats of each sex were given 0 or 2,500 ppm C.I. Direct Blue 15, 45 rats of each sex were given 630 ppm, and 75 rats of each sex were given 1,250 ppm. Interim evaluations were made at 9 and 15 months. The average amounts of compound consumed per day by the six dose groups after week 52 of the studies were estimated to be 45, 90, and 215 mg/kg for male rats and 50, 100, and 200 mg/kg for female rats.

Survival and Body Weights
The studies were terminated at 22 months due to extensive mortality associated with chemical-related neoplasia. Survival of control, 630, 1,250, and 2,500 ppm males at 22 months was 37/50, 8/35, 11/65, and 2/50; survival of females was 40/50, 13/35, 22/65, and 4/50. At 22 months, the mean final body weights of the 630, 1,250, and 2,500 ppm groups were 95%, 91%, and 81% of those of the control for male rats and 91% of those of the control for all female dose groups.

Histopathologic Effects in the 22-Month Studies
At the 9-month interim evaluations, one adenoma of the Zymbal's gland was seen in a high-dose male rat, and three carcinomas of the clitoral gland were seen in the high-dose females. At the 15-month interim evaluations, Zymbal's gland neoplasms were seen in low- and high-dose males and all treated female dose groups. Mid- and high-dose males and females also had preputial or clitoral gland neoplasms, and a few neoplasms were present in the skin, small and large intestine, liver, and oral cavity of treated animals at 15 months.

At the end of the study, neoplasms related to chemical administration were found in the Zymbal's gland, skin, oral cavity, and the preputial or clitoral gland in both male and female rats. Neoplasms related to chemical administration were also seen at other sites including the small and large intestine, liver, uterus, and brain. The incidence of mononuclear cell leukemia was also increased in treated rats. The incidences of these neoplasms are summarized in the table at the end of this section.

Genetic Toxicology
C.I. Direct Blue 15 was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 when tested in a standard preincubation protocol with or without exogenous metabolic activation; however, when a specialized reductive metabolism protocol was used, C.I. Direct Blue demonstrated mutagenic activity in *Salmonella* strain TA1538. C.I. Direct Blue 15 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.

Conclusions
Under the conditions of these 22-month drinking water studies, there was *clear evidence of carcinogenic activity** of C.I. Direct Blue 15 (desalted industrial grade) in male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity, and small and large intestine. Increased incidences of mononuclear cell leukemia and neoplasms of the brain may have been related to chemical administration.

There was *clear evidence of carcinogenic activity* of C.I. Direct Blue 15 in female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity, small and large intestine, and uterus, and by mononuclear cell leukemia.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of peer review comments and the public discussion on this Technical Report appear on page 11.

Summary of the Carcinogenesis and Genetic Toxicology Studies of C.I. Direct Blue 15

Male F344/N Rats	Female F344/N Rats
Drinking water concentration	
0, 630, 1,250, or 2,500 ppm C.I. Direct Blue 15	0, 630, 1,250, or 2,500 ppm C.I. Direct Blue 15
Body weights	
Dosed groups lower than controls during last 16 weeks of study	Dosed groups lower than controls during last 16 weeks of study
22-Month survival rates	
37/50, 8/35, 11/65, 2/50[a]	40/50, 13/35, 22/65, 4/50[a]
Nonneoplastic effects	
Preputial gland: ectasia: 5/49, 4/35, 15/64, 14/48	Clitoral gland: squamous hyperplasia: 0/50, 2/31, 4/64, 1/50
Liver: cystic degeneration: 1/50, 5/35, 10/61, 7/50	Liver: focal cellular alterations: 34/50, 18/35, 33/65, 23/50
Liver: focal cellular alterations: 27/50, 9/35, 19/61, 21/50	Liver: regeneration: 0/50, 0/35, 9/65, 7/50
Liver: regeneration: 1/50, 5/35, 4/61, 12/50	Zymbal's gland: ectasia: 1/49, 5/35, 13/64, 9/50
Zymbal's gland: ectasia: 2/50, 11/35, 8/64, 12/50	Zymbal's gland: hyperplasia: 0/49, 3/35, 4/64, 5/50
Zymbal's gland: squamous hyperplasia: 0/50, 1/35, 6/64, 5/50	
Neoplastic effects[b]	
Skin basal cell neoplasms: 2/50, 9/35, 27/65, 28/50	Skin squamous cell neoplasms: 0/50, 2/35, 6/65, 5/50
Skin sebaceous cell adenoma: 0/50, 1/35, 7/65, 3/50	Zymbal's gland neoplasms: 0/50, 4/35, 11/65, 17/50
Skin squamous cell neoplasms: 2/50, 4/35, 11/65, 19/50	Clitoral gland neoplasms: 7/50, 11/31, 24/64, 27/50
Zymbal's gland neoplasms: 1/50, 5/35, 10/65, 20/50	Hepatocellular neoplasms: 0/50, 0/35, 2/65, 5/50
Preputial gland neoplasms: 8/49, 5/35, 23/64, 9/48	Oral cavity neoplasms: 2/50, 4/35, 19/65, 15/50
Hepatocellular neoplasms: 0/50, 6/35, 9/65, 11/50	Small intestine adenocarcinoma: 0/50, 0/35, 1/65, 3/50
Oral cavity neoplasms: 1/50, 10/35, 24/65, 17/50	Large intestine adenomatous polyp: 0/50, 0/35, 3/65, 1/50
Small intestine neoplasms: 0/50, 1/35, 0/65, 2/50	Uterine epithelial neoplasms: 1/50, 0/35, 1/65, 4/50
Large intestine neoplasms: 0/50, 1/35, 6/65, 8/50	Mononuclear cell leukemia: 7/50, 13/35, 27/65, 15/50
Uncertain findings	
Mononuclear cell leukemia: 17/50, 19/35, 28/65, 20/50	
Brain neoplasms: 0/50, 1/35, 1/65, 2/50	
Level of evidence of carcinogenic activity	
Clear evidence	Clear evidence
Genetic toxicology	
Salmonella typhimurium gene mutation:	Negative with and without S9 in strains TA100, TA1535, TA1537, and TA 98
Salmonella typhimurium with reductive metabolism:	Positive in strain TA1538
Sister chromatid exchanges	
Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Chromosomal aberrations	
Chinese hamster ovary cells *in vitro*:	Negative with and without S9

[a] Reduced survival in exposed groups was due to neoplasia.
[b] Number of animals 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 Report 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 potency or mechanism.

- **Clear evidence** of carcinogenic activity describes 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 describes 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 describes studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity describes 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 is selected for a particular experiment, 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:

- 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);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- 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 draft technical report on C.I. Direct Blue 15 on November 19, 1990 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:

- 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 carcinogenic activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS

On November 19, 1990, the draft Technical Report on the toxicology and carcinogenesis studies of C.I. Direct Blue 15 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. Direct Blue 15 by discussing the uses of this chemical, describing the experimental design, and reviewing the 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. Dunnick explained that the studies were intended to last 24 months but were terminated after 22 months because of decreased survival of exposed animals due primarily to neoplasia.

Dr. Klaassen, a principal reviewer, agreed with the conclusions, but he wondered why the incidence of neoplasms rarely reached 50%. He questioned whether there was really an increased incidence of brain neoplasms in dosed female rats. Dr. Klaassen said his major concern was that the chemical was only about 50% pure and suggested that this be indicated in the title and elsewhere.

Dr. McKnight, the second principal reviewer, did not agree with the conclusions. She said the studies should be considered inadequate unless the impurities in the mixture could be characterized and listed and the study labeled as a test of industrial grade C.I. Direct Blue 15. Further, she thought the studies to be relevant only if it could be documented that the impurities were typical of those to which workers were exposed. Dr. McKnight stated that if these issues could be resolved, the highly statistically significant increases in mononuclear cell leukemias in male rats supported these lesions being included under *clear evidence*. She also suggested that the increased incidence of adrenal gland pheochromocytomas might be considered as part of the evidence in males. Dr. Dunnick responded that pheochromocytomas are commonly occurring neoplasms and there was no increase in the incidence of hyperplasias, which suggested a lack of association with chemical exposure. Dr. S.L. Eustis, NIEHS, agreed that mononuclear cell leukemia could be considered part of the evidence in male rats.

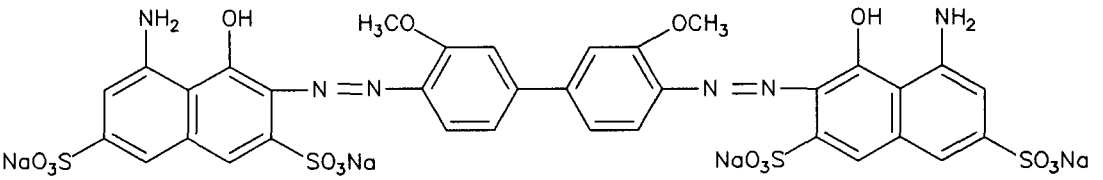
Dr. Zeise, the third principal reviewer, agreed with the conclusions. She shared the concern of the other reviewers about the composition of the chemical and the need to modify the title to reflect what was tested.

Responding to concerns of the reviewers about the purity of the chemical, Dr. Scala noted that the material studied was not industrial or technical grade but just C.I. Direct Blue 15. Dr. Dunnick said the sample was representative of what workers were exposed to, and more descriptive information would be added on the cover and inside. Additionally, information would be added on the two major impurities which are isomers of C.I. Direct Blue 15. Dr. McKnight pointed out that in the conclusions, the name of the chemical was followed by "desalted industrial grade" in parentheses. Dr. Dunnick said this would be added on the title page and cover. Dr. Silbergeld raised the question as to whether 3,3′-dimethoxybenzidine either formed metabolically or present as an impurity could be contributing to the neoplastic effects. Dr. Ashby agreed, noting that the dimethoxy compound would be formed *in vivo* by reductive cleavage, and pointing out that the only positive genetic toxicology finding was in *Salmonella* when reductive metabolism was incorporated.

Dr. Klaassen moved that the Technical Report on C.I. Direct Blue 15 be accepted with the revisions discussed, including wording about the purity and impurities present, and with the conclusions as written for male and female rats, *clear evidence of carcinogenic activity*. Dr. Goodman seconded the motion. Then, three amendments were offered and voted on. Dr. Klaassen moved that the last sentence of the conclusions for female rats be deleted, i.e., "Increased incidences of neoplasms of the brain may have been related to chemical administration." Dr. Goodman seconded the motion, which was accepted by nine yes to two no votes (Drs. Garman, Hayden) with one abstention (Dr. Ashby). Dr. McKnight moved that

mononuclear cell leukemias be added under *clear evidence* in male rats. Dr. Zeise seconded the motion, which was defeated by seven no votes (Drs. Ashby, Carlson, Gold, Goodman, Hayden, and Klaassen, with Chairman Scala breaking the tie) to six yes votes (Drs. Davis, Garman, Longnecker, McKnight, Silbergeld, Zeise). Dr. McKnight moved that adrenal pheochromocytomas be included in the conclusions for male rats as "may have been related to chemical administration." Dr. Silbergeld seconded the motion, which was defeated by nine no votes (Drs. Ashby, Carlson, Davis, Garman, Goodman, Gold, Hayden, Klaassen, Silbergeld) to three yes votes (Drs. Longnecker, McKnight, Zeise). The Panel then accepted the original motion unanimously with 12 votes.

INTRODUCTION



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USE AND PRODUCTION

C.I. Direct Blue 15 is a dark blue powder with a melting point of greater than 300° C. A benzidine congener-based dye, it is produced by coupling two moles of 1-amino-8-naphthol-3,6-disulfonic acid with one mole of 3,3′-dimethoxybenzidine dihydrochloride (*Colour Index*, 1956).

Azo dyes based on benzidine and benzidine congeners (3,3′-dimethylbenzidine dihydrochloride and 3,3′-dimethoxybenzidine dihydrochloride) constitute a group of over 90 dyes, all widely used in the United States. C.I. Direct Blue 15 is used as a dye to color textiles, paper, plastic, rubber, and leather (Fishbein, 1981).

The United States Environmental Protection Agency (USEPA) reports that there are seven manufacturers and one importer of C.I. Direct Blue 15 (USEPA, 1988). Although production volumes for three of the manufacturers and for the sole importer are listed as confidential, the remaining manufacturers reported production volumes collectively ranging from 0.1 to 1.1 million pounds. The most recent production volume data show that 270,000 pounds of C.I. Direct Blue 15 were produced in 1982 (USITC, 1983); the United States International Trade Commission (USITC) did not report domestic production volumes of C.I. Direct Blue 15 for 1985 or 1986 (USITC, 1986, 1987). In 1980, 7,716 pounds of the dye were imported (USITC, 1981).

EXPOSURE

From a survey conducted from 1981-1983, the National Institute for Occupational Safety and Health (NIOSH) has estimated that a total of

4,527 workers may be exposed to C.I. Direct Blue 15 (NIOSH, 1989). Industrial exposure to dyes may occur through inhalation of dust or mist, through accidental ingestion, or from direct contact of the dye with skin. The general public may be exposed to C.I. Direct Blue 15 through the use of home dye products or through contaminated water supplies (USEPA, 1980; Fishbein, 1981; NIOSH, 1983).

METABOLISM AND DISTRIBUTION

Reductive metabolism of 3,3′-dimethoxybenzidine-based dyes produces 3,3′-dimethoxybenzidine (Figure 1). Azo reduction can occur either in the liver via enzymes or in the gut by the 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 well (Walker, 1970). For this reason, reductive cleavage of the benzidine-congener azo dyes is thought to occur primarily through bacterial action in the gastrointestinal 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.

3,3′-Dimethoxybenzidine-based dyes, including C.I. Direct Blue 15, are metabolized to 3,3′-dimethoxybenzidine in dogs, rats, and humans (Lynn *et al.*, 1980; NIOSH, 1981). Azo dyes containing benzidine or one of its congeners can be reduced by mammalian liver azo reductases to form aromatic amines (Martin and Kennelly, 1981); rat intestinal microflora can also, through their azo reductases, metabolize the benzidine-based dyes to their aromatic amines (Cerniglia *et al.*, 1982). Urine recovered from dogs and rats given an oral dose of C.I. Direct Blue 15 contained primarily the N-acetyl derivatives of 3,3′-dimethoxybenzidine and small quantities of free 3,3′-dimethoxybenzidine. Genin (1977) also detected 3,3′-dimethoxybenzidine in the urine of rats exposed to dimethoxybenzidine-based dyes. In the same study, 3,3′-dimethoxybenzidine was detected in the urine of 3 of 22 workers who dried and ground two 3,3′-dimethoxybenzidine-based dyes. The metabolism of C.I. Direct Blue 15 to 3,3′-dimethoxybenzidine and subsequent metabolism of 3,3-dimethoxybenzidine (described by Rodgers *et al.*, 1983) is summarized in Figure 1.

When tissues from rats dosed orally with ^{14}C-C.I. Direct Blue 15 were analyzed for ^{14}C (Bowman *et al.*, 1982), peak tissue concentrations of ^{14}C were found in the brain, heart, lung, and small intestine at 4 hours after dosing, and in the urinary bladder, liver, kidney, lung, and carcass at 8 hours. The highest concentrations of ^{14}C were found in the liver, kidney, lung, and carcass.

Rodgers *et al.* (1983) reported that, after intravenous administration to male F344 rats, ^{14}C-3,3′-dimethoxybenzidine was rapidly and extensively metabolized, with less than 2% of the radiolabel recovered unchanged 30 minutes after dosing. Fifty percent of the radiolabel was located in the intestinal tract after 2 hours, and 70% was excreted in the bile within 72 hours. Three days after either oral or intravenous administration, 50% of the radiolabel had been excreted in the feces and 30% to 40% had been excreted in the urine; 45% of the radiolabel remaining in the animals was present in the liver in the form of covalently bound metabolites. Analysis of the pooled urine (days 0 to 3) demonstrated that more than 90% of the urinary radiolabel was in the form of metabolites, with unmetabolized 3,3′-dimethoxybenzidine dihydrochloride accounting for 3% to 9% of the urinary radiolabel and acetyldimethoxybenzidine accounting for 5% or less.

REPRODUCTIVE TOXICOLOGY

Wilson (1955) studied the teratogenic potential of several benzidine-based dyes in albino rats by injecting pregnant rats 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, followed by Evans blue, which caused abnormalities in 14%, Niagara blue 4B (C.I. Direct Blue 15), which caused abnormalities in 4%, and Niagara sky blue 6B, which caused abnormalities in 3%. 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). The abnormalities reported were generally similar to common spontaneous malformations such as anencephaly, hydrocephalus, and spina bifida. The purity and chemical characterization of the dyes used were not reported.

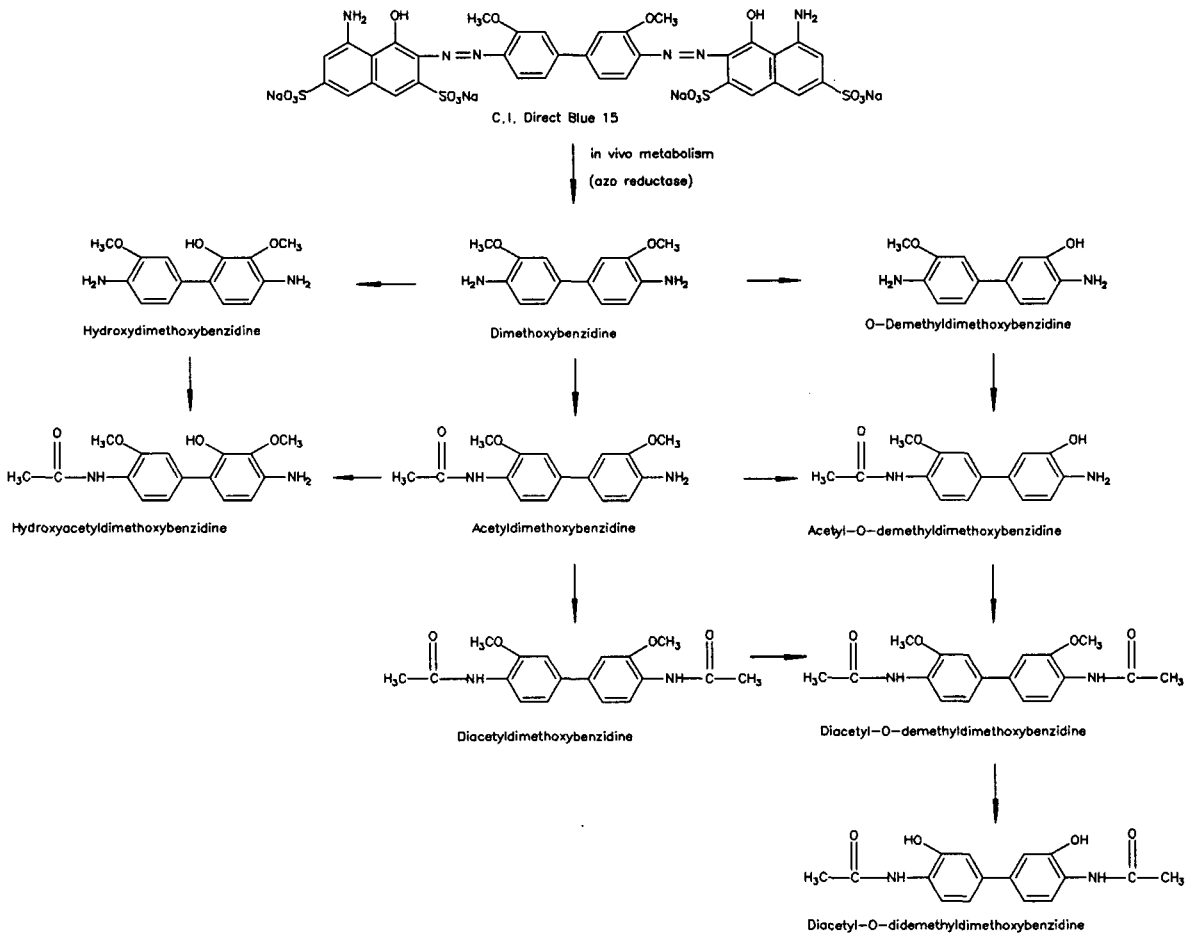


FIGURE 1
Proposed Metabolic Pathways of C.I. Direct Blue 15 and Dimethoxybenzidine (after Rodgers *et al.*, 1983)

TOXICITY AND CARCINOGENICITY STUDIES OF RELATED COMPOUNDS

In 1980, NIOSH and the Occupational Safety and Health Administration (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. Direct Blue 15 or 3,3′-dimethoxybenzidine in the absence of other suspected carcinogens were found in the literature.

Benzidine

C.I. Direct Blue 15 is a benzidine congener-based dye. Benzidine is a known carcinogen for humans (Scott, 1952; Case *et al.*, 1954; IARC, 1972; 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, 1972; Frith and Dooley, 1976). Occupational exposure to benzidine for up to 30 years resulted in urinary bladder neoplasms in as many as 90% of workers (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, 1972; Haley, 1975; USEPA, 1980; IARC, 1982).

Benzidine exposure caused urinary bladder neoplasms in dogs (Spitz *et al.*, 1950); hepatocellular, harderian gland, and lymphoreticular neoplasms 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.*, 1966). Animal survival was poor in many of the benzidine carcinogenicity studies. Although this was due in most cases to the administration of toxic doses, these studies demonstrated the carcinogenicity of benzidine in laboratory animals.

3,3′-Dimethoxybenzidine

In early rodent studies, repeated exposure to 3,3′-dimethoxybenzidine, the metabolite of C.I. Direct Blue 15, was shown to result in neoplasms of the gastrointestinal tract, Zymbal's gland, skin, and mammary gland of rats and hamsters (Pliss, 1963, 1965; Saffiotti *et al.*, 1966; Hadidian *et al.*, 1968). Although these early studies provided evidence that 3,3′-dimethoxybenzidine is carcinogenic, the use of small numbers of animals, the use of 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 and administration at 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.* (1966) fed diets containing 1,000 ppm 3,3′-dimethoxybenzidine to 30 male and female Syrian golden hamsters. After 144 weeks of exposure, the only neoplasm present 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 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 neo-

plasms occurred as early as day 293, most were detected at 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 in the treated rats was significantly increased over that in the 360 pooled vehicle and untreated control rats.

In the NTP dosed-water studies of 3,3′-dimethoxybenzidine dihydrochloride in rats, neoplasms of the skin, Zymbal's gland, preputial and clitoral glands, oral cavity, intestine, and liver, as well as mesotheliomas and brain neoplasms in males and neoplasms of the mammary gland and uterus in females were present (Table 1) (NTP, 1990a).

BALB/c mice were given 3,3′-dimethoxybenzidine dihydrochloride in drinking water at doses up to 630 ppm for 112 weeks. Body weight gain in mice that received 630 ppm was less than that of controls, but there was no evidence of neoplasms related to chemical administration in either sex (Schieferstein *et al.*, 1990).

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 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, the IARC (1972) concluded that 3,3′-dimethylbenzidine was a systemic carcinogen for rats when given subcutaneously. In the dosed-water studies in rats 3,3′-dimethylbenzidine caused neoplasms of the skin, Zymbal's gland, preputial and clitoral glands, oral cavity, intestine, liver, brain, and lung in male and female rats, and of the mammary gland and hematopoietic system in female rats (Table 1) (NTP, 1990b).

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

o-Anisidine

o-Anisidine (2-methoxyaniline), structurally analogous to one-half the 3,3′-dimethoxybenzidine molecule, is used to manufacture monoazo dyes by diazotization and coupling with other aromatic amines (Noller, 1965). The National Cancer Institute (NCI) found that *o*-anisidine was carcinogenic to F344/N rats and B6C3F$_1$ mice (NCI, 1978). Groups of 55 animals of each sex received 0, 5,000 or 10,000 ppm *o*-anisidine in feed for rats and 0, 2,500 or 5,000 ppm for mice for 103 weeks. Treatment with *o*-anisidine resulted in urinary bladder transitional cell carcinomas or papillomas in both sexes of each species. Male rats also had transitional cell carcinomas of the renal pelvis and follicular cell neoplasms of the thyroid gland. Only one animal in any of the control groups had a urinary system neoplasm, a transitional cell papilloma of the renal pelvis in a male mouse.

o-Toluidine

o-Toluidine (2-aminotoluene) is structurally analogous to one-half the 3,3′-dimethylbenzidine molecule. In NCI (1979) studies, *o*-toluidine hydrochloride was given to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex in feed at concentrations of 3,000 or 6,000 ppm for rats and 1,000 or 3,000 ppm for mice for 101 to 104 weeks. 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 hydrochloride 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.

TABLE 1
Summary of Results of Previous 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 2-Year Drinking Water Studies of C.I. Acid Red 114[c]	
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	
Skin sebaceous cell neoplasms: 1/50, 1/35, 5/65, 6/50	
Skin squamous cell neoplasms: 1/50, 2/35, 11/65, 9/50	
Zymbal's gland neoplasms: 0/50, 0/35, 8/65, 7/50	Zymbal's gland neoplasms: 0/50, 3/35, 18/65, 19/50
	Clitoral gland neoplasms: 11/48, 17/32, 28/62, 23/50
Liver neoplasms: 2/50, 2/35, 15/65, 20/50	Liver neoplasms: 0/50, 0/35, 19/64, 8/50
	Lung neoplasms: 1/50, 2/35, 9/65, 4/50
	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

[a] Dose groups: 0, 80, 170, 330 ppm
[b] Dose groups: 0, 30, 70, 150 ppm
[c] Dose groups: males: 0, 70, 150, 300 ppm; females: 0, 150, 300, 600 ppm

GENETIC TOXICOLOGY

Although information regarding the genotoxicity of C.I. Direct Blue 15 is limited, the available data from the testing of metabolites of C.I. Direct Blue 15 and of structurally related dyes corroborate the mutagenic potential of C.I. Direct Blue 15 after azoreduction and release of active metabolites. C.I. Direct Blue 15 has arylamine groupings, which are considered to be "structural alerts" for genotoxic activity (Ashby and Tennant, 1988), and, as with most benzidine-congener dyes, its activity in *Salmonella typhimurium* is dependent upon the presence of conditions that allow reductive metabolism of the azo bonds to release the parent amine. In standard *S. typhimurium* assays, C.I. Direct Blue 15 was not mutagenic with or without S9 (Mortelmans *et al.*, 1986); however, mutagenic activity was observed when reductive metabolism preceded incubation with the *S. typhimurium* tester strains TA98, TA100, and TA1538 (Gregory *et al.*, 1981; Brown and Dietrich, 1983; Prival *et al.*, 1984; Reid *et al.*, 1984a).

In the absence of specialized protocols for reductive metabolism, C.I. Direct Blue 15 has been tested in mammalian cell systems for induction of gene mutations in mouse L5178Y lymphoma cells (Rudd, 1983), chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells (Galloway *et al.*, 1987), and unscheduled DNA synthesis in Fischer 344/N rat hepatocyte cell cultures. All tests were negative except the gene mutation assay with L5178Y cells, which was positive in the presence of S9.

Results of genotoxicity tests with metabolites of C.I. Direct Blue 15 were largely positive. A key metabolite, 3,3′-dimethoxybenzidine, was positive in a variety of *in vitro* genotoxicity assays (NTP, 1990a). In NTP assays, 3,3′-dimethyoxybenzidine dihydrochloride induced gene mutations in *S. typhimurium* (Haworth *et al.*, 1983; Reid *et al.*, 1984a) and sister chromatid exchanges and chromosome aberrations in Chinese hamster ovary cells (Galloway *et al.*, 1985). It was negative for induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* (Yoon *et al.*, 1985). 3,3′-Dimethoxybenzidine is metabolized by various oxidative pathways to a variety of genotoxic compounds. For example, acetylation pathways produce N-acetyl-3,3′-dimethoxybenzidine, which is a more potent *S. typhimurium* mutagen than the parent compound or the diacetyl derivative (Rodgers *et al.*, 1983; Reid *et al.*, 1984a). Benzidine, the parent compound in this series of substituted biphenyls, was positive for induction of gene mutations in *S. typhimurium* with S9 (Haworth *et al.*, 1983; Reid *et al.*, 1984b), positive for induction of sister chromatid exchanges and chromosome aberrations in Chinese hamster ovary cells (Galloway *et al.*, 1987), and positive for induction of micronuclei, sister chromatid exchanges, and chromosome aberrations in bone marrow cells of mice exposed by intraperitoneal injection (NTP, unpublished data).

STUDY RATIONALE

Benzidine is a known human carcinogen (IARC, 1972, 1987), and the benzidine congeners, 3,3′-dimethylbenzidine dihydrochloride and 3,3′-dimethoxybenzidine dihydrochloride, are known animal carcinogens (NTP, 1990a,b). Since numerous benzidine and benzidine congener-based dyes are metabolized to these parent amines *in vivo* (Rinde and Troll, 1975; NCI, 1978; Lynn *et al.*, 1980; Nony *et al.*, 1980; Bowman *et al.*, 1982), all benzidine- and benzidine congener-derived dyes may be considered possible carcinogens. The dye C.I. Direct Blue 15 (desalted industrial grade) was selected for study as a representative of the dyes derived from 3,3'-dimethoxybenzidine, and the industrial product was used to examine the product to which humans are generally exposed.

NTP's Benzidine Dye Initiative is a collaborative effort of NIEHS, National Center for Toxicologic Research (NCTR), NIOSH, USEPA, the CPSC, and OSHA under the aegis of the NTP. The objective of the Initiative was to develop an integrated body of scientific data concerning the dyes derived from benzidine, 3,3′-dimethylbenzidine, and 3,3′-dimethoxybenzidine (Table 2). 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.

Five benzidine dyes were selected for toxicity and carcinogenicity studies: 3,3′-dimethoxybenzidine dihydrochloride and 3,3′-dimethylbenzidine dihydrochloride, which are benzidine congeners; C.I. Direct Blue 15 and C.I. Direct Blue 218, which are representative 3,3′-dimethoxybenzidine-based dyes; and

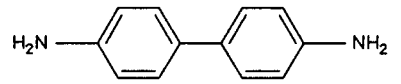
C.I. Acid Red 114, which is a representative 3,3′-dimethylbenzidine-based dye (Figure 2).

The oral route of administration was selected for these studies to mimic potential human exposure in the workplace and in the home. The NTP 2-year rat studies of 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine, and C.I. Acid Red 114 have been reported (NTP, 1990a,b; 1992). Long-term mouse studies of 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine, and other benzidine-based dyes have been conducted at NCTR. Auxiliary studies involved transplatation of neoplasms (Maronpot *et al.*, 1988 and Ulland *et al.*, 1989) and oncogene activation (Reynolds *et al.*, 1990).

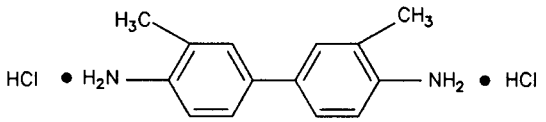
TABLE 2
Summary of the National Toxicology Program Benzidine Congener Initiative

Class/Chemical	Tests[a]
3,3′-Dimethylbenzidine (*o*-toluidine)	
o-Toluidine	G, P, B
C.I. Direct Red 2	G, M
C.I. Direct Red 39	G, M
C.I. Acid Red 114	G, P, B
C.I. Direct Blue 25	G
C.I. Direct Blue 53	G, M
C.I. Direct Blue 14	G
C.I. Direct Orange 6	G, M
3,3′-Dimethoxybenzidine (*o*-dianisidine)	
o-Dianisidine	G, P, B
C.I. Direct Blue 15	G, P, B
C.I. Direct Blue 218	G, P, B
C.I. Direct Black 114	G, M
C.I. Direct Yellow 68	G, M
C.I. Direct Blue 8	G, M

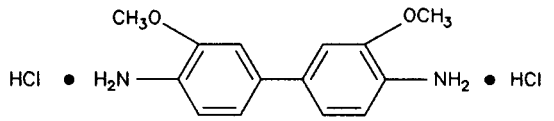
[a] G=genetic toxicology; P=pharmacokinetic studies; M=metabolism studies for detection of carcinogens in urine; B=toxicology and carcinogenicity studies.



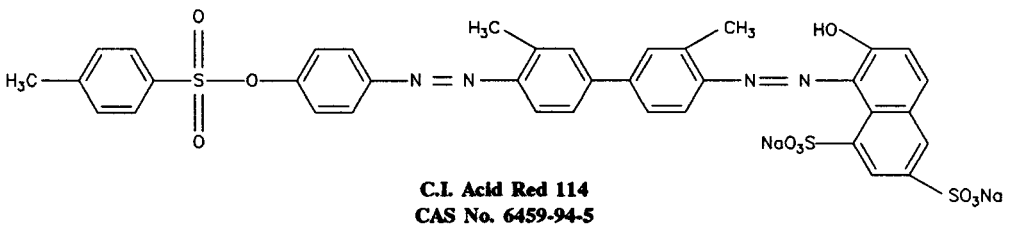
Benzidine
CAS No.92-87-5



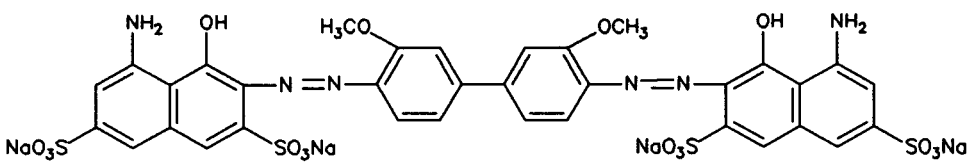
3,3'-Dimethylbenzidine Dihydrochloride
CAS No. 612-82-8



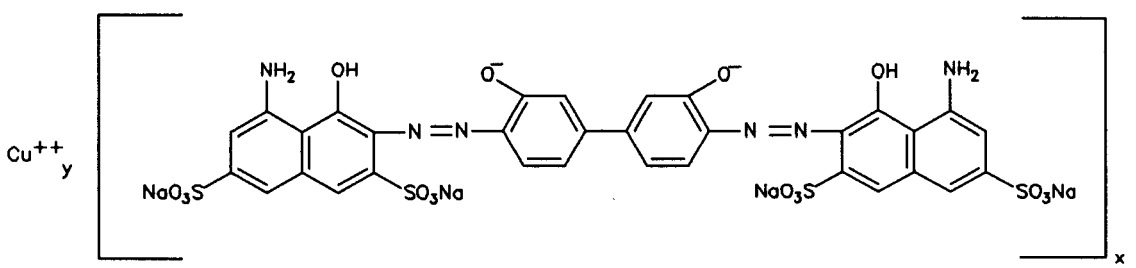
3,3'-Dimethoxybenzidine Dihydrochloride
CAS No. 20325-40-0



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 2
Chemical Structures of Benzidine and Selected Benzidine Congeners and Dyes

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF C.I. DIRECT BLUE 15

The dye, C.I. Direct Blue 15, was obtained from the Atlantic Chemical Company (Nutley, NJ) and supplied to the National Toxicology Program by Dyes Environmental and Toxicology Organization, Inc. (Scarsdale, NY) in two lots (NJ-0-62-611 and A03383-2). Because of the high salt content, the material was desalted by the analytical chemistry laboratory (Midwest Research Institute, Kansas City, MO). Lot NJ-0-62-611 was desalted in two batches, and the desalted material was assigned lot numbers M110481 and M042783. Lot A03383-2 was desalted and assigned lot number M080883. The resultant salt content was about 3%, reduced from approximately 25%. Lot number M110481 was used in the 14-day, 13-week, and 22-month studies, and lot numbers M042783 and M080883 were used in the 22-month studies. Purity, stability, and identity analyses were conducted on all lots at the analytical chemistry laboratory (Appendix F).

The study dye, a dark blue powder, was identified as C.I. Direct Blue 15 by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of all desalted lots was determined to be approximately 50% by combining the data from the following analyses: elemental analysis, Karl Fischer water analysis, azo group titrations, thin-layer chromatography, and high-performance liquid chromatography (HPLC). Comparison of the three lots by HPLC showed no significant purity differences. HPLC analysis detected approximately 35 impurities accounting for about 50% of the chromatographic peak area. The two largest imprints as shown by chromatographic analysis were identified by NMR spectrometry as related positional isomers to the major component. Each of the two largest imprints accounted for approximately 10% of the total sample by weight. Two of the lots (M042783 and M080883) were assayed for benzidine and 3,3′-dimethoxybenzidine dihydrochloride content. Benzidine was not detected in either batch at levels greater than 1 ppm, whereas 3,3′-dimethoxybenzidine dihydrochloride was found in lot M042783 at 836 ppm and in lot M080883 at 1,310 ppm. Stability studies performed with HPLC showed that C.I. Direct Blue 15 was stable as a bulk chemical for at least two weeks at temperatures up to 60° C when stored protected from light. Based on the stability study results, the bulk chemical was stored at room temperature in the dark at the study laboratory throughout the study period. The stability of the bulk chemical was monitored by the study laboratory using infrared spectroscopy, 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 appropriate amounts of C.I. Direct Blue 15 with tap water for the 14-day and 13-week studies, and with distilled water for the 22-month studies. Stability tests conducted by the analytical chemistry laboratory showed that solutions of 500 ppm C.I. Direct Blue 15 in water remained stable for at least 21 days when stored at room temperature. Solutions were stable for at least 3 days under simulated dosing conditions, including exposure to normal room light and air.

Dose formulations were prepared twice weekly and made available to the study animals on the day of mixing. The preparation and storage procedures for dosed drinking water in the studies of C.I. Direct Blue 15 are presented in Table F1.

The study laboratory analyzed the formulations used for dosing by ultraviolet spectroscopy at least once every 4 weeks during the 22-month studies. Based on the number of times the dose formulations were determined to be within 10% of the target concentration, it is estimated that 92% (104/113) of the formulations were prepared within specifications (Table F3). Results of periodic referee analyses by the analytical chemistry laboratory agreed with those of the study laboratory (Table F4).

14-DAY STUDIES

Male and female F344/N rats were obtained from Frederick Cancer Research Center (Frederick, MD) and observed for 14 days before the studies began. The rats were 50 days old when placed on study. Groups of five rats of each sex received 0, 1,250, 2,500, 5,000, 10,000, or 30,000 ppm C.I. Direct Blue 15 in drinking water for 14 consecutive days. Animals were housed five per cage, and water and feed were available *ad libitum*. Animals were observed twice daily. The animals were weighed at the start of the study and on days 7 and 14. Feed consumption was measured once weekly, and water consumption was measured twice weekly. All animals were necropsied, and the following organs were weighed: brain, heart, right kidney, liver, lung, right testis, and thymus. Complete histopathologic examinations were performed on all control animals and on males and females receiving 30,000 ppm. Selected tissues were examined from animals in the other dose groups. Further experimental details are presented in Table 3.

13-WEEK STUDIES

The 13-week studies were designed to evaluate the cumulative toxic effects of repeated exposure to C.I. Direct Blue 15 and to determine the chemical concentrations to be used in the 22-month studies.

Fischer 344/N rats were obtained from Frederick Cancer Research Center, observed for 21 days, distributed to weight classes, and assigned to dose groups. The rats were 56 days old when placed on study. Groups of ten rats of each sex received 0, 630 (females only), 500, 1,250, 2,500, 5,000, 10,000, or 30,000 (males only) ppm C.I. Direct Blue 15 in drinking water for 13 weeks. Rats were housed five per cage, and water and feed were available *ad libitum*. Animals were observed twice daily, and clinical observations were recorded weekly. Feed and water consumption were recorded by cage once weekly and twice weekly, respectively. Animals were weighed at the start of the study and weekly thereafter.

Blood was collected from all animals surviving to the end of the studies. Erythrocyte counts, leukocyte counts, differential leukocyte counts, hemoglobin concentrations, and hematocrit values were determined from samples drawn from the retro-orbital sinus. Clinical chemistry values for blood urea nitrogen, serum creatinine, lactic dehydrogenase, sorbitol dehydrogenase, and alanine aminotransferase were determined from blood samples collected from the abdominal aorta. Further details are presented in Table 3.

Survivors were killed at the end of the 13-week studies. All study animals were necropsied. The brain, heart, liver, lung, right kidney, right testis, and thymus were weighed at necropsy. Complete histopathologic examinations were performed on all animals in the control groups, all animals in the highest dose groups with a survival rate of 100% (10,000 ppm males and females), and all animals that died or were killed moribund (seven males from the 30,000 ppm group). Target organs were submitted for histopathology for the remaining animals. Tissues examined for each group are listed in Table 3.

22-MONTH STUDIES

Study Design

Rats received 0, 630, 1,250, or 2,500 ppm C.I. Direct Blue 15 in distilled drinking water for 96 weeks. There were 70 rats per control group, 45 rats per low-dose group, 75 rats per mid-dose group, and 70 rats per high-dose group. The 22-month studies were originally designed as 24-month studies with an animal allocation proposed by Portier and Hoel (1984). At 9 months, ten rats from the control and 2,500 ppm dose groups were killed, and at 15 months ten rats from each dose group were killed. Because of the high mortality in the dosed groups due to chemical-related neoplasia, the study was terminated at 22 months.

Source and Specification of Animals

Male and female F344/N rats were obtained from Simonsen Laboratories, Inc. (Gilroy, CA) for use in the 2-year studies. The animals were 4 weeks old at receipt. Following a 12- to 19-day quarantine, ten animals of each sex were randomly selected and killed for parasite evaluation and gross observation of disease. Blood samples were collected for viral screens. Study animals were 40 to 47 days old at study initiation. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

Animal Maintenance

The rats were housed five per cage. Feed and water were available *ad libitum*. Feed composition is presented in Appendix H. Further details of animal maintenance are given in Table 3. Information on cage rotation is not available.

Clinical Observations and Pathology

All animals were observed twice daily. Animals were weighed at study initiation, weekly for 16 weeks, and monthly thereafter. Clinical findings were recorded at the time of weighing. Feed consumption was measured weekly, and water consumption was measured twice weekly.

Blood and urine samples were collected from all interim evaluation animals. Hematocrit values, hemoglobin concentrations, erythrocyte counts, total leukocyte counts, leukocyte differential counts, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and blood cell morphology were determined from blood drawn from the retro-orbital sinus. Clinical chemistry values for blood urea nitrogen, creatinine, glucose, alanine aminotransferase, lactic dehydrogenase, sorbitol dehydrogenase, triiodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH), and serum osmolality were determined from blood samples collected from the abdominal aorta. T_3 and T_4 levels were analyzed with the Tri-Tab and Tetra-Tab Radioimmunoassay Diagnostic Kits (Nuclear Medical Laboratories). TSH values were determined by the method of Ridgway *et al.* (1973). Urine measurements included protein, glucose, creatinine, pH, specific gravity, urine osmolality, volume, and creatine excretion rate (16-hour); urine sediment was examined microscopically. Brain, liver, and kidney weights were measured at necropsy. Further details are presented in Table 3.

Animals found moribund, designated for the 9- or 15-month interim evaluations, or surviving to the end of the 22-month studies were killed. All animals were necropsied. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, trimmed and processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. The tissues and dose groups examined are listed in Table 3.

When the pathology evaluation was completed by the study laboratory pathologist, and the pathology data were 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. At this 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 neoplasms and nonneoplastic lesions were reviewed in the following tissues from all male and female rats: liver, lung (males only), small intestine, large intestine, Zymbal's gland, preputial gland, clitoral gland, and uterus. Spleens and livers from all males and females were reviewed for 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 in 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 treatment-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 liver, lung, small and large intestine, spleen, Zymbal's gland, preputial or clitoral gland, skin, pharynx, tongue, and uterus and examples of disagreements in diagnosis between the laboratory and quality assessment pathologist were shown to the PWG. The PWG, which included the quality assessment pathologist and others experienced in rodent toxicologic pathology, 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 Cox's method (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 that were necropsied.

Analysis of Neoplasm Incidence

In the 22-month studies, the deaths of dosed rats and rats killed moribund were considered to be due primarily to neoplasms of the Zymbal's gland, preputial gland, clitoral gland, and skin, and possibly to mononuclear cell leukemia. Consequently, for these lesions, primary emphasis in the analysis of neoplasm incidence was given to the life table test (Cox, 1972; Tarone, 1975), a survival-adjusted procedure appropriate for rapidly lethal neoplasms.

For incidental neoplasms (neoplasms discovered as a result of death from an unrelated cause), the primary statistical method used in these studies was logistic regression, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm 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 neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These methods include the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the effective number of animals (i.e., the number of animals surviving until the appearance of the first neoplasm).

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 neoplasm incidence. 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 neoplasm incidence. Although the current studies were terminated at 22 months, neoplasm incidences from the NTP historical control database for 2-year studies (Haseman *et al.*, 1984, 1985) are included for neoplasms appearing to show compound-related effects.

Analysis of Continuous Variables

Clinical chemistry, urinalysis and hematology data, and organ and body weights 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 (Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response trend (Dunn's test). For the 9-month interim evaluations (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, clinical chemistry, and urinalysis data.

Quality Assurance Methods

The 13-week and 22-month 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 preliminary review 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 discrepancies had been resolved or were otherwise addressed during the preparation of this Technical Report.

TABLE 3
Experimental Design and Materials and Methods in the Drinking Water Studies of C.I. Direct Blue 15

14-Day Studies	13-Week Studies	22-Month 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)	Simonsen Laboratories, Inc. (Gilroy, CA)
Time Held Before Study 14 days	21 days	12-19 days
Average Age When Placed on Study 50 days	56 days	40-47 days
Date of First Dose 11 March 1982	1 June 1982	28 February 1983
Duration of Dosing 14 consecutive days	13 weeks (7 days/week)	96 weeks (7 days/week)
Date of Last Dose 25 March 1982	31 August 1982	30 December 1984
Average Age at Necropsy 9 weeks	21 weeks	103-104 weeks 46/47 weeks (9-month interim) 72/73 weeks (15-month interim)
Necropsy Dates 25 March 1982	1 and 3 September 1982	7-10 January 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 14-day studies	Same as 14-day studies
Animals per Cage 5	5	5
Method of Animal Identification Ear tag	Ear punch	Ear tag

TABLE 3
Experimental Design and Materials and Methods in the Drinking Water Studies of C.I. Direct Blue 15
(continued)

14-Day Studies	13-Week Studies	22-Month Studies
Diet		
NIH-07 Rat and Mouse Ration, powdered (Zeigler Bros., Inc., Gardners, PA), available *ad libitum*	Same as 14-day studies	Same as 14-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*	Same as 14-day studies	Distilled water (Polar Water Co., Beltsville, MD) in glass water bottles with stainless steel sippers (Hazleton Systems, Inc., Aberdeen, MD), available *ad libitum*
Cages		
Polycarbonate (Hazleton Systems, Inc., Aberdeen, MD)	Same as 14-day studies	Same as 14-day studies
Bedding		
Heat-treated hardwood chips (P.J. Murphy Forest Products, Mt. Jewett, PA)	Same as 14-day studies	Same as 14-day studies
Cage Filters		
Reemay nonwoven polyester fiber filters (DuPont Company, Applied Technologies Division, Wilmington, DE)	Same as 14-day studies	Same as 14-day studies
Animal Room Environment		
Temperature: 71°-74° F Relative humidity: 19%-69% Fluorescent light: 12 hours/day	Temperature: 70°-75° F Relative humidity: 33%-79% Fluorescent light: 12 hours/day Room air changes: 10-12/hour	Temperature: 67°-82° F Relative humidity: 22%-87% Fluorescent light: 12 hours/day Room air changes: 12.1/hour
Doses		
0, 1,250, 2,500, 5,000, 10,000 or 30,000 ppm C.I. Direct Blue 15 in drinking water	0, 630 (females only), 1,250, 2,500, 5,000, 10,000, or 30,000 ppm (males only) C.I. Direct Blue 15 in drinking water	0, 630, 1,250, or 2,500 ppm C.I. Direct Blue 15 in distilled drinking water
Type and Frequency of Observation		
Observed twice daily; body weight initially and weekly; feed consumption weekly; water consumption twice weekly; clinical observation weekly	Observed twice daily; body weight initially and weekly; feed consumption weekly; water consumption twice weekly; clinical observation weekly	Observed twice daily; body weights initially, weekly for 16 weeks, and monthly thereafter; feed consumption measured 1 week every 4 weeks; water consumption measured in a 3- or 4-day segment every 4 weeks; clinical observations at body weight determinations

TABLE 3
Experimental Design and Materials and Methods in the Drinking Water Studies of C.I. Direct Blue 15
(continued)

14-Day Studies	13-Week Studies	22-Month Studies
Necropsy All animals necropsied. Organ weights obtained at necropsy (brain, heart, liver, lung, right kidney, right testis, and thymus).	**Necropsy** All animals necropsied. Organ weights measured were the same as in the 14-day studies.	**Necropsy** All animals necropsied. Organ weights measured at 9-month and 15-month interim sacrifices (brain, kidney, liver).
Histopathology Complete histopathology on male and female control and high-dose (30,000 ppm) animals, including the following organs: adrenal gland, blood smear, bone (sternebrae, femur, or vertebrae, including marrow), brain, clitoral gland, epididymis, esophagus, eyes (if grossly abnormal), heart, kidney, large intestines (cecum, colon, rectum), liver, lung with mainstem bronchi, lymph nodes (mandibular, mesenteric), nasal turbinates, ovaries, pancreas, parathyroid gland, pharynx (if grossly abnormal), pituitary gland, preputial gland, prostate gland, salivary gland, small intestines (duodenum, ileum, jejunum), spinal cord (if neurological signs present), spleen, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus, Zymbal's gland, and gross lesions. The following organs were examined from 10,000 ppm males: liver; from 10,000 ppm females: kidneys, liver, and thymus; and from 5,000 and 2,500 ppm females: kidneys.	**Histopathology** Complete histopathology on male and female controls, all males and females receiving 10,000 ppm, and all deaths and moribund kills (7 males from the 30,000 ppm group). Tissues examined were the same as in the 14-day studies complete screen. The following organs were examined from 5,000 ppm males: kidney and thymus; from 5,000 ppm females: kidney; and from 1,250 and 2,500 ppm males: kidney.	**Histopathology** Complete histopathology on all animals that died, were killed moribund, were killed at 9 months or 15 months (control and high-dose animals only), or were killed at study termination. Tissues examined were the same as in the 14-day studies complete screen, with the addition of seminal vesicles. Organs for low-dose and mid-dose animals killed at 15 months included the liver, preputial and clitoral glands, and Zymbal's gland.
Clinical Pathology None required	**Clinical Pathology** Clinical pathology studies conducted at the end of the studies. ***Hematology:*** hematocrit, hemoglobin, erythrocytes, leukocyte count and differential ***Clinical chemistry:*** blood urea nitrogen, creatinine, lactate dehydrogenase, sorbitol dehydrogenase, alanine aminotransferase	**Clinical Pathology** Clinical pathology studies 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:*** blood urea nitrogen, creatinine, glucose, serum osmolality, triiodothyronine, thyroxine, thyroid stimulating hormone, lactate dehydrogenase, sorbitol dehydrogenase, alanine aminotransferase ***Urinalyses:*** Protein, glucose, creatinine, pH, specific gravity, urine osmolality, volume, creatinine excretion rate (16 hr), serum/urine osmolality ratio, microscopic exam of sediment

RESULTS

14-DAY STUDIES

All rats survived to the end of the studies (Table 4). The final mean body weight of females receiving 30,000 ppm was 34% lower than that of controls; final mean body weights of other dosed groups were within 11% of the respective control groups. Water consumption declined with increasing dose, and animals in the 30,000 ppm groups consumed less than half that consumed by control animals. The eyes, skin, and feces of all treated rats were stained blue, and high-dose females were thin or emaciated.

There were no notable necropsy findings, although organs and tissues were stained blue in all high-dose animals and in decreasing numbers of lower dose animals. Males receiving 10,000 or 30,000 ppm had increased absolute and relative kidney weights. Females receiving 30,000 ppm showed decreased absolute and relative thymus weights (Tables E1 and E2). Treatment-related histologic changes were seen in the liver and kidney of high-dose male and female rats and in the thymus of high-dose females. Liver lesions included necrosis of individual hepatocytes in males and females and mild degeneration of centrilobular hepatocytes in females. Blue granular pigment was present in renal tubule epithelial cells in both sexes. In addition, mild to moderate renal tubule degeneration was seen in females, affecting multiple scattered tubules and characterized by severe swelling of epithelial cells often accompanied by nuclear pyknosis. Moderate depletion of thymic lymphocytes also occurred in high-dose females.

TABLE 4
Survival, Mean Body Weights, and Water Consumption of Rats in the 14-Day Drinking Water Studies of C.I. Direct Blue 15

Concentration (ppm)	Survival[a]	Mean Body Weights[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	162 ± 4.9	207 ± 5.4	+45 ± 2.8		42	39
1,250	5/5	164 ± 4.5	229 ± 3.6	+66 ± 1.8	111	41	33
2,500	5/5	165 ± 4.6	205 ± 4.8	+41 ± 1.9	99	40	42
5,000	5/5	161 ± 4.1	224 ± 3.2	+62 ± 1.7	108	35	34
10,000	5/5	157 ± 3.6	220 ± 5.9	+63 ± 3.5	106	38	38
30,000	5/5	157 ± 4.1	191 ± 5.2	+34 ± 1.9	92	20	20
Female							
0	5/5	128 ± 1.1	149 ± 1.8	+21 ± 2.7		40	36
1,250	5/5	130 ± 1.5	161 ± 3.3	+31 ± 2.2	108	32	29
2,500	5/5	130 ± 1.7	159 ± 2.5	+29 ± 1.1	107	32	30
5,000	5/5	129 ± 2.1	156 ± 2.7	+27 ± 0.9	105	24	28
10,000	5/5	129 ± 2.3	157 ± 3.5	+28 ± 1.7	105	24	29
30,000	5/5	126 ± 2.9	99 ± 7.0	-27 ± 7.7**	66	12	13

** Significantly different (P≤0.01) from the control group by Dunn's test
[a] Number surviving/number initially in group
[b] Weights and weight changes given as mean ± standard error
[c] Milliliters per animal per day, based on average consumption data per group per week for weeks 1 and 2

13-WEEK STUDIES

Seven males in the 30,000 ppm group died before the end of the study (Table 5); there were no other deaths. The final mean body weight of surviving males receiving 30,000 ppm was 31% lower than controls. The final mean body weights in the other dosed male and female groups were 92% to 98% of those of the corresponding controls. Clinical findings included hunched posture, rough hair coat, depression, and thin appearance in high-dose males. The skin of dosed animals was stained blue.

Mean absolute and relative kidney weights were significantly increased in both sexes receiving 5,000 and 10,000 ppm, and in surviving males receiving 30,000 ppm (Tables E3 and E4). Significant differences in relative mean organ weights occurred in various dose groups and were considered to be due to the decreased body weights.

Female rats showed statistically significant increases in mean erythrocyte count, hematocrit, absolute lymphocyte count, and blood urea nitrogen (Table D1). These findings were consistent with mild hemoconcentration.

All tissues of males receiving 30,000 ppm were stained blue, and blue staining of the mesenteric lymph nodes and intestinal contents was noted in males and females in all but the lowest dose groups. Treatment-related lesions were seen in the kidneys and livers of males that received 30,000 ppm and in the kidneys of males and females that received 10,000 ppm (Table 6). Renal tubule regeneration occurred in nearly all males, including controls, but occurred with increased severity in males that received 10,000 or 30,000 ppm. Minimal tubule regeneration occurred in treated females, but not in controls, and generally resembled the change seen as a part of the chronic nephropathy that occurs commonly in F344/N rats. Regeneration consisted of tubules lined by a few small cuboidal cells or by increased numbers of cuboidal cells with basophilic cytoplasm and hyperchromatic nuclei; some regenerative tubules were shrunken and surrounded by thickened basement membranes. Lesions of minimal severity involved only scattered tubules, usually affecting less than 10% of all tubules. Mild lesions involved up to 25% and moderate lesions up to 50% of the tubules. Necrosis of individual tubule epithelial cells (tubule necrosis), characterized by nuclear pyknosis, cytoplasmic eosinophilia, and sloughing of necrotic cells into the tubule lumen, was seen in scattered tubules in several 30,000 ppm males. A few males given 30,000 ppm also had bits of mineral within some tubule lumens (mineralized tubules). Blue staining of tubule epithelial cells (tubule pigmentation), presumably due to the presence of the dye, occurred in a few males in the 10,000 and 30,000 ppm dose groups. Tubule degeneration was seen in several females that received 10,000 ppm and was characterized by scattered tubules containing enlarged epithelial cells with abundant finely vacuolated cytoplasm that often filled most of the tubule lumen.

A variety of treatment-related liver changes was observed in six of the high-dose males. These changes included minimal enlargement of periportal hepatocytes (megalocytosis), and degeneration, fatty metamorphosis, or necrosis of centrilobular hepatocytes. Degeneration of centrilobular hepatocytes consisted of individual cells that were smaller with more eosinophilic cytoplasm than normal cells but with normal appearing nuclei. In some animals the degeneration of hepatocytes appeared to proceed to individual cell necrosis. Fatty metamorphosis was characterized by multiple variably sized clear vacuoles (lipid) within the cytoplasm. Blue pigment was seen within Kupffer cells lining hepatic sinusoids adjacent to centrilobular areas. Most of the males in the 30,000 ppm dose group also demonstrated loss of small to moderate numbers of lymphocytes from the thymus gland (lymphoid depletion).

Dose Selection Rationale

Because of the presence of dose-related kidney and liver lesions and increased relative kidney weights, drinking water concentrations of 0, 630, 1,250, and 2,500 ppm C.I. Direct Blue 15 were selected for rats in the 22-month studies.

22-MONTH STUDIES

9-Month Interim Evaluation

At 2,500 ppm, males showed a statistically significant increase in relative liver weight and females an increase in absolute kidney weight (Tables E5 and E6). Various hematology and chemistry parameters were significantly different from the

TABLE 5
Survival, Mean Body Weights, and Water Consumption of Rats in the 13-Week Drinking Water Studies of C.I. Direct Blue 15

Concentration (ppm)	Survival[a]	Mean Body Weights[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 12
Male							
0	10/10	163 ± 3.2	353 ± 4.5	+190 ± 2.6		23	19
1,250	10/10	164 ± 3.1	329 ± 5.0**	+165 ± 3.2**	93	21	28
2,500	10/10	163 ± 3.5	339 ± 7.5*	+176 ± 5.5**	96	22	23
5,000	10/10	159 ± 3.4	335 ± 3.8*	+176 ± 1.8**	95	18	30
10,000	10/10	169 ± 2.5	325 ± 3.6**	+156 ± 3.5**	92	16	28
30,000	3/10[d]	164 ± 2.9	244 ± 32.1**	+75 ± 30.3**	69	6	16
Female							
0	10/10	130 ± 1.7	205 ± 3.2	+74 ± 2.0		34[e]	21
630	10/10	128 ± 2.7	196 ± 2.5	+68 ± 2.2	96	23	29
1,250	10/10	131 ± 1.3	201 ± 2.0	+70 ± 1.1	98	25	25
2,500	10/10	131 ± 1.7	201 ± 1.9	+70 ± 1.7	98	18	24
5,000	10/10	129 ± 1.7	198 ± 2.3	+69 ± 1.6	97	17	24
10,000	10/10	131 ± 2.4	193 ± 2.2**	+62 ± 1.2**	94	15	19

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test
** $P \leq 0.01$
[a] Number surviving/number initially in group
[b] Body weights and body weight changes given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study. Differences from the control group are not significant by Dunn's or Shirley's test.
[c] Milliliters per animal per day, based on average consumption data per group per week for weeks 1 and 12
[d] Week of death: 3, 3, 4, 5, 10, 11, 13
[e] Empty water bottle one weighing

TABLE 6
Incidences of Selected Treatment-Related Lesions in Rats in the 13-Week Drinking Water Studies of C.I. Direct Blue 15

Male	0 ppm	10,000 ppm	30,000 ppm
Kidney[a]			
Tubule regeneration	10/10 (1.0)[b]	10/10 (1.4)	6/7 (2.7)
Tubule necrosis	0/10	0/10	5/7**
Tubule pigment	0/10	4/10*	4/7*
Mineralized tubules	0/10	0/10	3/7
Liver			
Megalocytosis	0/10	0/10	4/7*
Fatty metamorphosis	0/10	0/10	5/7**
Hepatocyte degeneration	0/10	0/10	6/7**
Individual hepatocyte necrosis	0/10	0/10	6/7**
Pigment	0/10	0/10	6/7**
Thymus			
Lymphoid depletion	0/10	0/10	5/7**
Female	**0 ppm**	**5,000 ppm**	**10,000 ppm**
Kidney			
Tubule regeneration	0/10	1/10	9/10** (1.1)
Tubule degeneration	0/10	0/10	5/10*

* Significantly different ($P \leq 0.05$) from the control group by Fisher's exact test
** $P \leq 0.01$
[a] Kidneys of male rats receiving 1,250 ppm or 5,000 ppm were also examined for tubule regeneration, necrosis, pigment, and mineralized tubules; lesion incidences and severity were the same as for the control group.
[b] Severity grade. Severity of 1=minimal, 2=mild, 3=moderate.

control group in males, including a decreased 16-hour mean urine volume with a high mean specific gravity and increased mean osmolality, osmolality ratio, and creatinine (Table D2). Females showed decreases in mean erythrocyte count and hematocrit, indicative of slight anemia. No differences in other parameters were considered biologically significant. Significant histopathologic findings included a Zymbal's gland adenoma in 1/10 high-dose (2,500 ppm) males and clitoral gland carcinomas in 3/10 high-dose (2,500 ppm) females.

15-Month Interim Evaluation

Clinical findings included the appearance of tissue masses beginning at week 40. Mean body weights at necropsy were significantly less than controls in the high-dose males and females (Tables E7 and E8). Males that received 1,250 and 2,500 ppm showed statistically significant increased absolute and relative liver weights. Statistically significant increases in relative organ weight occurred in various organs and dose groups and were considered to be due to decreases in body weight. Few hematology and clinical chemistry parameters varied significantly from controls (Table D3). High-dose males had a decreased hematocrit and mean cell volume, increased absolute segmented neutrophil count, and decreased absolute lymphocyte and eosinophil count; females in the same dose group had a decreased hemoglobin concentration. Both males and females had statistically significant decreased T_4 values. Serum creatinine was decreased in all female dose groups. Urinalysis results for high-dose males were similar to those found in males at the 9-month interim evaluation; these changes were not duplicated in the female dose groups. However, rats in all treated groups showed increased urine pH.

A variety of neoplasms and nonneoplastic lesions related to chemical administration were found in male and female rats administered C.I. Direct Blue 15 for 15 months (Table 7). Lesions sites included the Zymbal's gland, preputial or clitoral gland, skin, oral cavity, intestine, and liver.

Body Weights, Water Consumption, and Clinical Findings

At 22 months, the final mean body weights of the 630, 1,250, and 2,500 ppm groups were 95%, 91%, and 81% of controls for males and 91% of controls for all female groups (Figure 3 and Tables 8 and 9). From week 52 to 97, the average daily water consumption per male rat in the low-, mid-, and high-dose groups was 9%, 9%, and 24% higher than that by the controls; for exposed female rats the consumption was 15%, 17%, and 12% higher than that by the controls. The average amount of C.I. Direct Blue 15 consumed per rat per day during weeks 51 through 97 was approximately 45, 90, and 215 mg/kg for low-, mid, and high-dose males and 50, 100, and 200 mg/kg for low-, mid-, and high-dose females (Tables G1 and G2). Clinical findings were limited to the appearance of tissue masses and swellings in the ventral body, in the genital region, and on the head, which usually corresponded to skin or mammary lesions, preputial/clitoral masses, and Zymbal's gland neoplasms seen at necropsy. General pallor and emaciation were also noted in treated animals.

TABLE 7
Incidences of Selected Treatment-Related Lesions in Rats at the 15-Months Interim Evaluation of the 22-Month Drinking Water Studies of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Male				
Liver				
Hepatocholangiocarcinoma	0/10	0/10	0/10	1/10
Neoplastic nodule	0/10	1/10	0/10	0/10
Eosinophilic focus	0/10	2/10	0/10	6/10**
Zymbal's Gland				
Carcinoma	0/10	1/10	0/10	2/10
Adenoma	0/10	0/10	0/10	1/10
Hyperplasia, focal	0/10	0/10	0/10	1/10
Preputial Gland				
Carcinoma	0/10	0/10	4/10*	1/10
Adenoma	0/10	0/10	0/10	2/10
Hyperplasia, focal	0/10	0/10	2/10	1/10
Oral Cavity (Tongue or Pharynx)				
Papilloma, squamous cell	0/10	3/10	0/10	0/10
Skin				
Basal cell carcinoma	0/10	0/10	2/10	1/10
Papilloma, squamous cell	0/10	1/10	0/10	2/10
Large Intestine				
Adenomatous polyp	0/10	1/10	1/10	2/10
Small Intestine				
Adenocarcinoma	0/10	0/10	0/10	1/10
Female				
Liver				
Neoplastic nodule	1/10	0/10	0/10	0/10
Eosinophilic focus	0/10	0/10	0/10	1/10
Zymbal's Gland				
Adenoma	0/10	2/10	1/10	3/10
Hyperplasia, squamous	0/10	2/10	1/10	0/10
Clitoral Gland				
Carcinoma	0/10	0/10	2/10	1/10
Adenoma	1/10	1/10	1/10	1/10
Hyperplasia, focal	0/10	0/10	1/10	1/10
Oral Cavity (Pharynx)				
Papilloma, squamous cell	0/10	0/10	0/10	2/10
Large Intestine				
Adenomatous polyp	0/10	0/10	0/10	1/10
Small Intestine				
Adenocarcinoma	0/10	0/10	0/10	1/10

* Significantly different ($P \leq 0.05$) from the control group by Fisher's exact test
** $P \leq 0.01$

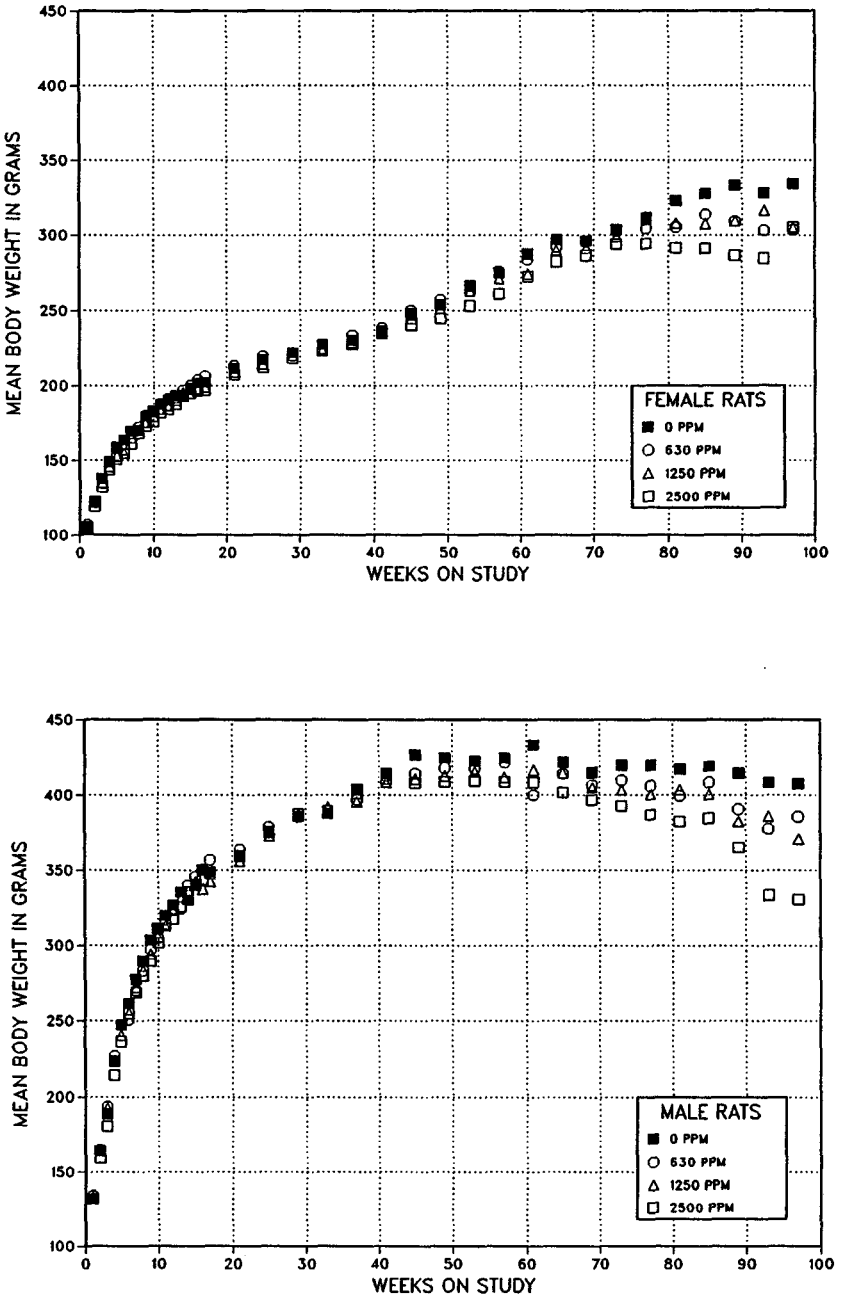


FIGURE 3
Growth Curves for Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

TABLE 8
Mean Body Weights and Survival of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

Week on Study	0 ppm		630 ppm			1,250 ppm			2,500 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	131	50	134	102	35	134	102	65	132	101	50
2	162	50	166	102	35	164	101	65	159	98	50
3	191	50	197	103	35	193	101	65	180	94	50
4	222	50	227	102	35	223	100	65	215	97	50
5	246	50	248	101	35	240	97	65	235	96	50
6	260	50	249	96	35	254	98	65	254	98	50
7	277	50	270	98	35	272	98	65	268	97	50
8	290	50	285	98	35	283	98	65	279	96	50
9	302	50	297	98	35	294	97	65	289	96	50
10	311	50	304	98	35	305	98	65	301	97	50
11	319	50	317	100	35	312	98	65	313	98	50
12	325	50	324	99	35	323	99	65	316	97	50
13	334	50	323	97	35	330	99	65	324	97	50
14	329	50	340	103	35	337	103	65	333	101	48
15	339	50	345	102	35	342	101	65	338	100	48
16	349	50	350	100	35	337	97	65	339	97	48
17	347	50	356	103	35	343	99	65	344	99	48
21	357	50	363	102	35	356	100	65	356	100	48
25	374	50	376	101	35	372	100	65	370	99	48
29	385	50	382	99	35	388	101	65	385	100	48
33	388	50	386	100	35	392	101	65	387	100	48
37	401	50	391	98	35	395	99	65	397	99	47
41	414	50	410	99	35	407	98	65	408	99	47
45	426	50	409	96	34	410	96	65	405	95	47
49	422	49	412	98	33	411	97	64	407	96	46
53	421	48	413	98	33	415	98	64	405	96	46
57	422	48	417	99	33	410	97	63	405	96	43
61	431	48	395	92	32	414	96	62	405	94	42
65	420	47	410	98	31	413	98	62	398	95	42
69	415	47	406	98	31	405	98	59	397	96	36
73	420	45	410	98	31	403	96	56	393	94	31
77	420	45	406	97	29	401	95	54	387	92	27
81	417	44	400	96	28	404	97	52	382	92	20
85	419	43	408	97	23	400	96	44	385	92	14
89	415	42	391	94	20	382	92	40	365	88	10
93	408	40	377	92	15	386	95	22	334	82	8
97	407	37	386	95	8	371	91	13	331	81	2
Mean for weeks											
1-13	259		257	100		256	99		251	97	
14-52	377		377	100		374	99		372	99	
53-97	418		402	96		400	96		382	91	

TABLE 9
Mean Body Weights and Survival of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

Week on Study	0 ppm		630 ppm			1,250 ppm			2,500 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	106	50	106	101	35	105	99	65	104	98	50
2	123	50	122	99	35	122	99	65	120	97	50
3	138	50	134	97	35	135	98	65	132	96	50
4	149	50	149	100	35	146	98	65	144	96	50
5	159	50	159	100	35	153	96	65	151	95	50
6	164	50	155	94	35	156	95	65	157	96	50
7	170	50	169	100	35	167	98	65	161	95	50
8	170	50	172	102	35	171	101	65	168	99	50
9	179	50	181	101	35	176	98	65	173	97	50
10	183	50	184	101	35	180	98	65	176	97	50
11	187	50	189	101	35	185	99	65	182	97	50
12	190	50	192	101	35	187	99	65	185	97	50
13	193	50	192	100	35	191	99	65	188	98	50
14	195	50	198	102	35	196	101	65	193	99	50
15	198	50	201	101	35	198	100	65	195	99	50
16	200	50	205	102	35	198	99	65	196	98	50
17	201	50	206	103	35	199	99	65	197	98	50
21	211	50	213	101	35	210	99	65	207	98	50
25	216	50	220	102	35	214	99	65	213	98	50
29	222	50	220	99	35	223	101	65	219	99	50
33	227	50	225	99	35	224	99	65	224	99	50
37	230	50	234	102	35	229	100	64	228	99	50
41	236	50	239	101	35	236	100	64	235	100	50
45	247	49	250	101	35	245	99	64	241	98	49
49	253	49	258	102	35	252	100	64	245	97	48
53	265	49	263	99	35	264	100	64	254	96	46
57	274	49	276	101	35	272	99	64	262	96	45
61	286	49	283	99	35	273	96	60	274	96	42
65	295	49	291	99	35	291	99	57	285	97	42
69	296	49	295	100	33	291	98	56	286	97	38
73	303	47	302	100	32	299	99	54	294	97	32
77	312	47	304	98	28	310	100	51	294	94	29
81	323	46	305	95	25	308	95	49	292	90	27
85	328	46	314	96	21	307	94	48	291	89	21
89	333	46	309	93	21	309	93	44	287	86	15
93	328	45	303	92	18	316	97	33	284	87	8
97	334	41	303	91	13	304	91	25	305	91	4
Mean for weeks											
1-13	162		162	100		160	98		157	97	
14-52	220		222	101		219	100		216	98	
53-97	306		296	97		295	97		284	93	

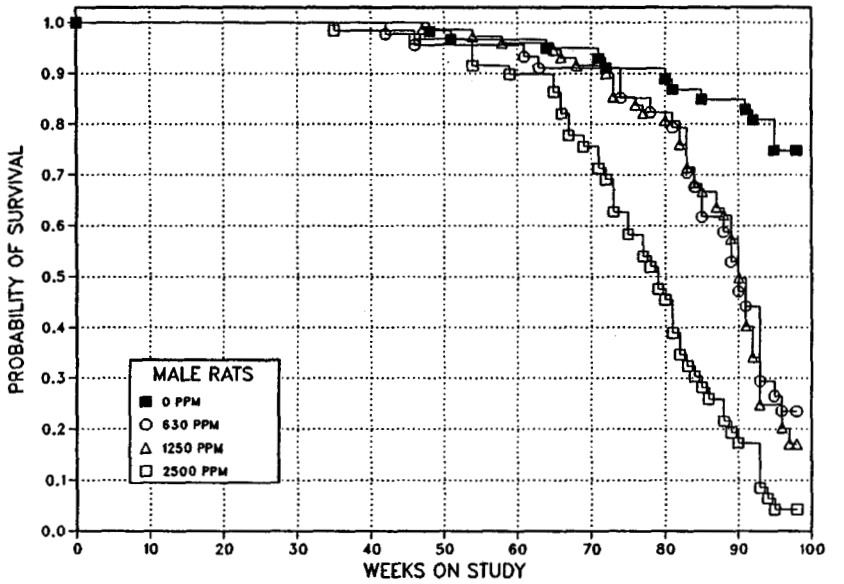
Survival

Estimates of the probabilities of survival for male and female rats given C.I. Direct Blue 15 and for controls are shown in Table 10 and in the Kaplan-Meier curves in Figure 4. By week 81, almost half of the high-dose males and females had been found dead or were killed while moribund from chemical-induced neoplasia.

TABLE 10
Survival of Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Male[a]				
Animals initially in study	70	45	75	70
9-Month interim evaluation	10	0	0	10
15-Month interim evaluation	10	10	10	10
Natural deaths	5	12	24	12
Moribund kills	8	15	30	34
Accidental deaths				2
Animals surviving until study termination	37	8	11	2
Percent survival at end of studies[b]	75	24	17	4
Mean survival (days)[c]	632	565	584	472
Survival analyses[d]	P<0.001	P<0.001	P<0.001	P<0.001
Female[a]				
Animals initially in study	70	45	75	70
9-Month interim evaluation	10	0	0	10
15-Month interim evaluation	10	10	10	10
Natural deaths	4	4	12	15
Moribund kills	6	18	31	31
Animals surviving until study termination	40	13	22	4
Percent survival at end of studies[b]	80	37	35	8
Mean survival (days)[c]	662	577	587	493
Survival analyses[d]	P<0.001	P<0.001	P<0.001	P<0.001

[a] First day of terminal kill: male, 680; female, 682
[b] Kaplan-Meier determinations. Survival rates adjusted for accidental deaths and interim evaluations.
[c] Mean of all deaths (uncensored, censored, terminal kill).
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns.



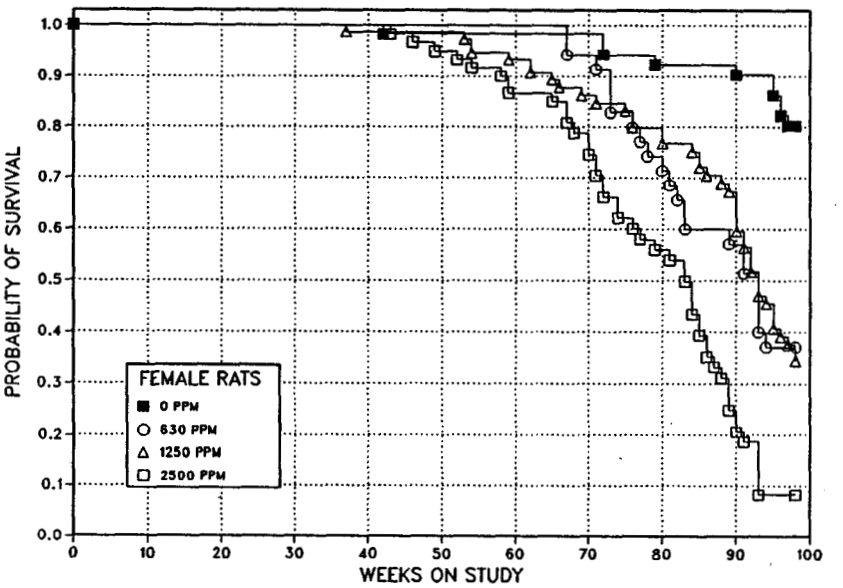


FIGURE 4
Kaplan-Meier Survival Curves for Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences in rats of neoplasms or nonneoplastic lesions of the skin, Zymbal's gland, clitoral and preputial glands, hematopoietic system, liver, oral cavity (tongue or pharynx), small intestine, large intestine, uterus, brain, kidney, adrenal gland, spleen, bone marrow, and heart.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms 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: The incidences of a variety of epithelial neoplasms of the skin were increased in male and female rats treated with C.I. Direct Blue 15 (Table 11). The incidences of basal cell adenomas, basal cell carcinomas, and basal cell adenomas or carcinomas (combined) were moderately increased in low-dose males and markedly increased in mid- and high-dose males. Many of the treated males had multiple basal cell adenomas. There was no increase in the incidence of basal cell neoplasms in treated females. Several sebaceous gland adenomas occurred in treated males, but not in control males (Plate 1); the incidence of this lesion was significantly increased in the mid- and high-dose groups. The incidence of squamous cell papilloma was significantly increased in high-dose males and mid- and high-dose females, while the incidence of squamous cell carcinoma was significantly increased in mid- and high-dose males only. The incidence of squamous cell papilloma or squamous cell carcinoma (combined) was significantly increased in the mid- and high-dose groups of each sex.

Basal cell neoplasms were composed of small, basophilic cells that formed sheets, cords, or solid lobules sometimes containing 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. Some neoplasms consisted solely of sebaceous elements and were diagnosed as sebaceous gland adenoma or carcinoma. Squamous cell papillomas were exophytic growths composed of a pedunculated, highly branched fibrovascular core covered by thickened stratified squamous epithelium. Squamous cell carcinomas were highly invasive neoplasms consisting of irregular cords of disordered pleomorphic squamous cells that projected into the dermis and often showed varying degrees of keratin formation.

Zymbal's Gland: Zymbal's glands are specialized sebaceous glands that lie ventral and anterior to the orifice of the external ear. The incidence of Zymbal's gland neoplasms was markedly increased in treated male and female rats (Table 12). The incidence of adenomas or carcinomas (combined) was significantly increased in all treated groups of males and females. Zymbal's glands from some treated animals of each sex contained nonneoplastic changes, including focal hyperplasia of the glandular cells, squamous focal hyperplasia of the squamous epithelium lining glandular ducts, and dilatation of ducts.

There was a morphologic continuum from adenoma to carcinoma. Adenomas were discrete nodular masses composed of glandular acini of relatively normal-looking 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. Often atypical, neoplastic cells exhibited disordered growth patterns and formed solid masses, irregular acinar structures, and cords with scattered ductular structures and areas of necrosis. Some carcinomas consisted principally of sebaceous cells, while others were composed mainly of stratified squamous epithelium; some neoplasms had prominent components of both. A few of the carcinomas metastasized to the lung or lymph node.

TABLE 11
Skin Proliferative Lesions in Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Male				
Basal Cell Hyperplasia				
Overall rates[a]	1/50 (2%)	1/35 (3%)	3/65 (5%)	3/50 (6%)
Basal Cell Adenoma				
Overall rates	2/50 (4%)	8/35 (23%)	23/65 (35%)	26/50 (52%)
Effective rates[b]	2/48 (4%)	8/33 (24%)	23/62 (37%)	26/43 (60%)
Terminal rates[c]	1/37 (3%)	2/8 (25%)	8/11 (73%)	2/2 (100%)
First incidence (days)	659	632	460	408
Life table tests[d]	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests[d]	P<0.001	P=0.001	P<0.001	P<0.001
Basal Cell Carcinoma				
Overall rates	0/50 (0%)	2/35 (6%)	4/65 (6%)	10/50 (20%)
Effective rates	0/45 (0%)	2/28 (7%)	4/53 (8%)	10/23 (43%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	2/2 (100%)
First incidence (days)	-[e]	646	637	551
Life table tests	P<0.001	P=0.063	P=0.018	P<0.001
Logistic regression tests	P<0.001	P=0.122	P=0.065	P<0.001
Basal Cell Adenoma or Carcinoma[f]				
Overall rates	2/50 (4%)	9/35 (26%)	27/65 (42%)	28/50 (56%)
Effective rates	2/48 (4%)	9/33 (27%)	27/62 (44%)	28/43 (65%)
Terminal rates	1/37 (3%)	2/8 (25%)	8/11 (73%)	2/2 (100%)
First incidence (days)	659	632	460	408
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P<0.001	P<0.001	P<0.001
Sebaceous Gland Adenoma				
Overall rates	0/50 (0%)	1/35 (3%)	7/65 (11%)	3/50 (6%)
Effective rates	0/44 (0%)	1/28 (4%)	7/52 (13%)	3/21 (14%)
Terminal rates	0/37 (0%)	1/8 (13%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	680 (T)	633	561
Life table tests	P<0.001	P=0.200	P<0.001	P=0.001
Logistic regression tests	P=0.002	P=0.200	P=0.004	P=0.026
Squamous Cell Papilloma				
Overall rates	2/50 (4%)	3/35 (9%)	5/65 (8%)	8/50 (16%)
Effective rates	2/47 (4%)	3/31 (10%)	5/61 (8%)	8/40 (20%)
Terminal rates	2/37 (5%)	1/8 (13%)	2/11 (18%)	1/2 (50%)
First incidence (days)	680 (T)	578	617	460
Life table tests	P<0.001	P=0.087	P=0.018	P<0.001
Logistic regression tests	P=0.001	P=0.258	P=0.107	P=0.005
Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	7/65 (11%)	13/50 (26%)
Effective rates	0/47 (0%)	1/31 (3%)	7/61 (11%)	13/40 (33%)
Terminal rates	0/37 (0%)	0/8 (0%)	2/11 (18%)	1/2 (50%)
First incidence (days)	-	591	460	539
Life table tests	P<0.001	P=0.380	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.461	P=0.018	P<0.001

TABLE 11
Skin Proliferative Lesions in Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Male (continued)				
Squamous Cell Papilloma or Squamous Cell Carcinoma[g]				
Overall rates	2/50 (4%)	4/35 (11%)	11/65 (17%)	19/50 (38%)
Effective rates	2/47 (4%)	4/31 (13%)	11/61 (18%)	19/40 (48%)
Terminal rates	2/37 (5%)	1/8 (13%)	3/11 (27%)	1/2 (50%)
First incidence (days)	680 (T)	578	460	460
Life table tests	P<0.001	P=0.034	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.146	P=0.011	P<0.001
Female				
Basal Cell Adenoma				
Overall rates	1/50 (2%)	0/35 (0%)	0/65 (0%)	0/50 (0%)
Basal Cell Adenoma or Carcinoma[h]				
Overall rates	1/50 (2%)	0/35 (0%)	1/65 (2%)	0/50 (0%)
Squamous Cell Papilloma				
Overall rates	0/50 (0%)	2/35 (6%)	5/65 (8%)	5/50 (10%)
Effective rates	0/47 (0%)	2/28 (7%)	5/51 (10%)	5/28 (18%)
Terminal rates	0/40 (0%)	1/13 (8%)	4/22 (18%)	0/4 (0%)
First incidence (days)	-	535	673	607
Life table tests	P<0.001	P=0.087	P=0.005	P<0.001
Logistic regression tests	P=0.001	P=0.199	P=0.007	P=0.003
Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	1/65 (2%)	0/50 (0%)
Squamous Cell Papilloma or Squamous Cell Carcinoma[i]				
Overall rates	0/50 (0%)	2/35 (6%)	6/65 (9%)	5/50 (10%)
Effective rates	0/47 (0%)	2/28 (7%)	6/51 (12%)	5/28 (18%)
Terminal rates	0/40 (0%)	1/13 (8%)	4/22 (18%)	0/4 (0%)
First incidence (days)	-	535	666	607
Life table tests	P<0.001	P=0.087	P=0.002	P<0.001
Logistic regression tests	P=0.001	P=0.199	P=0.004	P=0.003

(T)Terminal kill
[a] Number of tumor-bearing animals/number of animals necropsied
[b] Number of tumor-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 control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between 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] Not applicable; no tumors in animal group
[f] Historical incidence for 2-year NTP studies of untreated control groups (mean ± standard deviation): 21/1596 (1.3% ± 1.9%)
[g] Historical incidence: 29/1596 (1.8% ± 1.7%)
[h] Historical incidence: 6/1643 (0.4% ± 0.8%)
[i] Historical incidence: 7/1643 (0.4% ± 0.8%)

TABLE 12
Zymbal's Gland Proliferative Lesions in F344/N Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Male				
Squamous Hyperplasia				
Overall rates[a]	0/50 (0%)	1/35 (3%)	6/64 (10%)	5/50 (10%)
Adenoma				
Overall rates	0/50 (0%)	2/35 (6%)	2/65 (3%)	4/50 (8%)
Effective rates[b]	0/45 (0%)	2/28 (7%)	2/53 (4%)	4/23 (17%)
Terminal rates[c]	0/37 (0%)	1/8 (13%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-[e]	660	577	551
Life table tests[d]	P<0.001	P=0.023	P=0.228	P=0.004
Logistic regression tests[d]	P=0.024	P=0.054	P=0.316	P=0.041
Carcinoma				
Overall rates	1/50 (2%)	3/35 (9%)	8/65 (12%)	17/50 (34%)
Effective rates	1/50 (2%)	3/33 (9%)	8/65 (12%)	17/46 (37%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	501	583	323	372
Life table tests	P<0.001	P=0.128	P=0.014	P<0.001
Logistic regression tests	P<0.001	P=0.247	P=0.083	P=0.001
Adenoma or Carcinoma[f]				
Overall rates	1/50 (2%)	5/35 (14%)	10/65 (15%)	20/50 (40%)
Effective rates	1/50 (2%)	5/33 (15%)	10/65 (15%)	20/46 (43%)
Terminal rates	0/37 (0%)	1/8 (13%)	0/11 (0%)	0/2 (0%)
First incidence (days)	501	583	323	372
Life table tests	P<0.001	P=0.007	P=0.005	P<0.001
Logistic regression tests	P<0.001	P=0.045	P=0.037	P<0.001

TABLE 12
Zymbal's Gland Proliferative Lesions in F344/N Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Female				
Glandular or Squamous Hyperplasia				
Overall rates	0/49 (0%)	3/35 (9%)	4/64 (6%)	5/50 (10%)
Adenoma				
Overall rates	0/50 (0%)	1/35 (3%)	5/65 (8%)	3/50 (6%)
Effective rates	0/49 (0%)	1/35 (3%)	5/60 (8%)	3/42 (7%)
Terminal rates	0/40 (0%)	0/13 (0%)	1/22 (5%)	0/4 (0%)
First incidence (days)	-	495	432	547
Life table tests	P=0.006	P=0.421	P=0.016	P=0.031
Logistic regression tests	P=0.118	P=0.462	P=0.067	P=0.122
Carcinoma				
Overall rates	0/50 (0%)	4/35 (11%)	7/65 (11%)	14/50 (28%)
Effective rates	0/49 (0%)	4/35 (11%)	7/64 (11%)	14/50 (28%)
Terminal rates	0/40 (0%)	0/13 (0%)	1/22 (5%)	1/4 (25%)
First incidence (days)	-	465	432	296
Life table tests	P<0.001	P=0.017	P=0.007	P<0.001
Logistic regression tests	P=0.001	P=0.056	P=0.037	P=0.001
Adenoma or Carcinoma[g]				
Overall rates	0/50 (0%)	4/35 (11%)	11/65 (17%)	17/50 (34%)
Effective rates	0/49 (0%)	4/35 (11%)	11/64 (17%)	17/50 (34%)
Terminal rates	0/40 (0%)	0/13 (0%)	2/22 (9%)	1/4 (25%)
First incidence (days)	-	465	432	296
Life table tests	P<0.001	P=0.017	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.056	P=0.004	P<0.001

[a] Number of tumor-bearing animals/number of animals necropsied or examined microscopically for this tumor type
[b] Number of tumor-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 control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between 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] Not applicable; no tumors in animal group
[f] Historical incidence for 2-year NTP studies of untreated control groups (mean ± standard deviation): 18/1596 (1.1% ± 1.8%)
[g] Historical incidence: 14/1643 (0.9% ± 1.5%)

Clitoral and Preputial Glands: The clitoral glands of the female rat are bilateral modified sebaceous glands located near the base of the clitoris. The preputial glands of the male rat are homologous organs located near the penis. There was a marked treatment-related increase in the incidence of clitoral gland neoplasms in female rats (Table 13). The incidence of clitoral gland adenomas or carcinomas (combined) was significantly increased in all treated female groups, and many treated females developed bilateral adenomas or carcinomas. The incidence of preputial gland adenomas or carcinomas (combined) was significantly increased only in the mid-dose male group (Table 14; Plate 2). The incidences of nonneoplastic changes of the clitoral or preputial glands were higher in treated rats than in controls. The incidence of hyperplasia of the stratified squamous epithelium lining glandular ducts was slightly increased in treated females, and the incidence of dilatation of the ducts (ectasia) was moderately increased incidence in mid- and high-dose male rats.

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 with a few duct-like structures, sometimes containing debris, scattered within the neoplasms. Carcinomas were poorly demarcated masses that sometimes invaded adjacent tissues. They were composed of solid sheets and clusters of disorganized pleomorphic cells, and there was often an abundance of small, basophilic basal-like cells (reserve cells). Some carcinomas exhibited marked cellular atypia or contained large areas of necrosis.

Hematopoietic System: The incidence of mononuclear cell leukemia was significantly increased in all treated male and female groups (Table 15) as determined by survival-adjusted analyses. The incidence in the high-dose groups was somewhat less than that in the mid-dose groups, perhaps because of the reduced survival and competing risks from other fatal neoplasms.

TABLE 13
Clitoral Gland Lesions in Female F344/N Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Squamous Hyperplasia				
Overall rates[a]	0/50 (0%)	2/31 (6%)	4/64 (6%)	1/50 (2%)
Adenoma				
Overall rates	5/50 (10%)	5/31 (16%)	12/64 (19%)	12/50 (24%)
Effective rates[b]	5/49 (10%)	5/31 (16%)	12/59 (20%)	12/42 (29%)
Terminal rates[c]	4/40 (10%)	3/13 (23%)	5/22 (23%)	2/4 (50%)
First incidence (days)	666	558	432	453
Life table tests[d]	P<0.001	P=0.074	P=0.007	P<0.001
Logistic regression tests[d]	P=0.003	P=0.197	P=0.077	P=0.006
Carcinoma				
Overall rates	2/50 (4%)	6/31 (19%)	12/64 (19%)	15/50 (30%)
Effective rates	2/50 (4%)	6/31 (19%)	12/64 (19%)	15/50 (30%)
Terminal rates	2/40 (5%)	1/13 (8%)	3/22 (14%)	0/4 (0%)
First incidence (days)	682 (T)	506	253	372
Life table tests	P<0.001	P=0.010	P=0.002	P<0.001
Logistic regression tests	P=0.025	P=0.057	P=0.063	P=0.005
Adenoma or Carcinoma[e]				
Overall rates	7/50 (14%)	11/31 (35%)	24/64 (38%)	27/50 (54%)
Effective rates	7/50 (14%)	11/31 (35%)	24/64 (38%)	27/50 (54%)
Terminal rates	6/40 (15%)	4/13 (31%)	8/22 (36%)	2/4 (50%)
First incidence (days)	666	506	253	372
Life table tests	P<0.001	P=0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.028	P=0.010	P<0.001

(T)Terminal kill
[a] Number of tumor-bearing animals/number of animals examined microscopically for this tumor type
[b] Number of tumor-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 control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between 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 NTP studies of untreated control groups (mean ± standard deviation): 115/1643 (7.0% ± 4.9%)

TABLE 14
Preputial Gland Neoplasms in Male F344/N Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Adenoma				
Overall rates[a]	6/49 (12%)	2/35 (6%)	12/64 (19%)	8/48 (17%)
Effective rates[b]	6/47 (13%)	2/33 (6%)	12/63 (19%)	8/44 (18%)
Terminal rates[c]	5/37 (14%)	1/8 (13%)	4/11 (36%)	0/2 (0%)
First incidence (days)	565	660	530	372
Life table tests[d]	P<0.001	P=0.560	P=0.002	P<0.001
Logistic regression tests[d]	P=0.039	P=0.466N	P=0.143	P=0.228
Carcinoma				
Overall rates	2/49 (4%)	3/35 (9%)	11/64 (17%)	1/48 (2%)
Effective rates	2/47 (4%)	3/33 (9%)	11/63 (17%)	1/44 (2%)
Terminal rates	2/37 (5%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	680 (T)	578	372	600
Life table tests	P=0.092	P=0.107	P=0.003	P=0.379
Logistic regression tests	P=0.441N	P=0.300	P=0.056	P=0.687
Adenoma or Carcinoma[e]				
Overall rates	8/49 (16%)	5/35 (14%)	23/64 (36%)	9/48 (19%)
Effective rates	8/47 (17%)	5/33 (15%)	23/63 (37%)	9/44 (20%)
Terminal rates	7/37 (19%)	1/8 (13%)	4/11 (36%)	0/2 (0%)
First incidence (days)	565	578	372	372
Life table tests	P<0.001	P=0.141	P<0.001	P<0.001
Logistic regression tests	P=0.121	P=0.547	P=0.019	P=0.232

(T)Terminal kill
[a] Number of tumor-bearing animals/number of animals examined microscopically for this tumor type
[b] Number of tumor-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 control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between 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 dose group is indicated by N.
[e] Historical incidence for 2-year NTP studies of untreated control groups (mean ± standard deviation): 117/1596 (7.3% ± 5.2%)

TABLE 15
Leukemias in F344/N Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Male[a]				
Overall rates[b]	17/50 (34%)	19/35 (54%)	28/65 (43%)	20/50 (40%)
Effective rates[c]	17/48 (35%)	19/31 (61%)	28/62 (45%)	20/42 (48%)
Terminal rates[d]	11/37 (30%)	5/8 (63%)	9/11 (82%)	2/2 (100%)
First incidence (days)	445	544	472	452
Life table tests[e]	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests[e]	P=0.004	P=0.018	P=0.053	P=0.012
Female[f]				
Overall rates	7/50 (14%)	13/35 (37%)	27/65 (42%)	15/50 (30%)
Effective rates	7/49 (14%)	13/35 (37%)	27/58 (47%)	15/42 (36%)
Terminal rates	5/40 (13%)	3/13 (23%)	11/22 (50%)	2/4 (50%)
First incidence (days)	624	463	449	453
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.006	P=0.025	P<0.001	P<0.001

[a] Historical incidence for 2-year drinking water studies with untreated control groups (mean ± standard deviation): 594/1,596 (37% ± 16%); range 10%-72%
[b] Number of tumor-bearing animals/number of animals necropsied
[c] Number of tumor-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 control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between 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 drinking water studies with untreated control groups (mean ± standard deviation): 324/1,643 (20% ± 8%); range 6%-40%

Liver: The incidence of neoplastic nodules or hepatocellular carcinoma (combined) was significantly increased in all treated male groups and in the high-dose female group (Table 16). Livers of a few treated males contained multiple neoplastic nodules. No hepatocellular neoplasms occurred in control animals of either sex. Neoplastic nodule is the term previously used for proliferative hepatocellular lesions currently classified as hepatocellular adenoma. Neoplastic nodules were well-demarcated masses that compressed the adjacent parenchyma and varied in size from several hepatic lobules to nearly an entire liver lobe. The hepatic plates within neoplastic nodules were not organized in a normal lobular pattern and often intersected at nearly right angles with the plates of the adjacent normal liver. In some cases, sinusoids were apparent within neoplastic nodules, but generally the nodules appeared to be more solid than the surrounding parenchyma. Neoplastic hepatocytes were slightly pleomorphic and exhibited increased eosinophilic staining. Hepatocellular carcinomas, in contrast, consisted of highly disorganized cells that formed solid clusters, glandular structures, and broad trabeculae many cell layers thick. Cells within carcinomas were often moderately to markedly pleomorphic and exhibited varying degrees of atypia.

A variety of nonneoplastic liver lesions were present in treated male and female rats (Table 17). The incidence of eosinophilic foci was moderately to markedly increased in all treated groups of males and slightly increased in the high-dose female group. 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. The incidences of hematopoietic cell proliferation and regeneration were slightly to moderately increased in treated male and female rats. Hematopoietic cell proliferation was presumably secondary to inflammation associated with neoplasms in treated animals. Regeneration was characterized by one or more discrete nodular foci consisting of increased numbers of hepatocytes with normal morphology arranged in a lobular pattern. Regeneration represents an attempt by the liver to recover from hepatocellular injury. The increase in the incidence of regeneration in this study was considered secondary to hepatocellular damage caused by mononuclear cell leukemia, which was more common in treated animals. The incidence of degenerative changes was marginally increased in treated males and females. These changes included single or multiple small scattered foci of hepatocyte necrosis (most commonly affecting centrilobular hepatocytes), the presence of clear cytoplasmic vacuoles in scattered clusters of hepatocytes (cytoplasmic vacuolization), and multiple focal clusters of variably sized cysts filled with granular eosinophilic materials or erythrocytes (cystic degeneration).

TABLE 16
Liver Neoplasms in F344/N Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Male				
Neoplastic Nodule				
Overall rates[a]	0/50 (0%)	6/35 (17%)	8/65 (12%)	7/50 (14%)
Effective rates[b]	0/47 (0%)	6/31 (19%)	8/60 (13%)	7/38 (18%)
Terminal rates[c]	0/37 (0%)	3/8 (38%)	2/11 (18%)	0/2 (0%)
First incidence (days)	-[e]	544	579	463
Logistic regression tests[d]	P=0.003	P=0.002	P=0.003	P=0.003
Hepatocellular Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	1/65 (2%)	4/50 (8%)
Effective rates	0/45 (0%)	0/28 (0%)	1/53 (2%)	4/24 (17%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	-	632	547
Logistic regression tests	P<0.001	-	P=0.540	P=0.009
Neoplastic Nodule or Hepatocellular Carcinoma[f]				
Overall rates	0/50 (0%)	6/35 (17%)	9/65 (14%)	11/50 (22%)
Effective rates	0/47 (0%)	6/31 (19%)	9/60 (15%)	11/38 (29%)
Terminal rates	0/37 (0%)	3/8 (38%)	2/11 (18%)	0/2 (0%)
First incidence (days)	-	544	579	463
Logistic regression tests	P<0.001	P=0.002	P=0.002	P<0.001
Female				
Neoplastic Nodule				
Overall	0/50 (0%)	0/35 (0%)	2/65 (3%)	4/50 (8%)
Effective	0/46 (0%)	0/21 (0%)	2/48 (4%)	4/24 (17%)
Terminal	0/40 (0%)	0/13 (0%)	1/22 (5%)	1/4 (25%)
First incidence (days)	-	-	625	585
Logistic regression tests	P=0.002	-	P=0.246	P=0.016
Hepatocellular Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	0/65 (0%)	1/50 (2%)
Neoplastic Nodule or Hepatocellular Carcinoma[g]				
Overall	0/50 (0%)	0/35 (0%)	2/65 (3%)	5/50 (10%)
Effective	0/46 (0%)	0/25 (0%)	2/49 (4%)	5/27 (19%)
Terminal	0/40 (0%)	0/13 (0%)	1/22 (5%)	1/4 (25%)
First incidence (days)	-	-	625	564
Logistic regression tests	P<0.001	-	P=0.246	P=0.010

[a] Number of tumor-bearing animals/number of animals necropsied
[b] Number of tumor-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 control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard tumors in animals dying prior to terminal kill as nonfatal.
[e] Not applicable; no tumors in animal group
[f] Historical incidence for 2-year NTP studies of untreated control groups (mean ± standard deviation): 78/1591 (4.9% ± 4.3%)
[g] Historical incidence: 37/1643 (2.3% ± 2.7%)

TABLE 17
Numbers of F344/N Rats with Selected Nonneoplastic Liver Lesions in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

Lesion	Male				Female			
	0 ppm	630 ppm	1,250 ppm	2,500 ppm	0 ppm	630 ppm	1,250 ppm	2,500 ppm
n	50	35	65	50	50	35	65	50
Eosinophilic focus	2	7 *	15 **	23 ***	2	2	3	6
Hematopoietic cell proliferation	0	4 *	6 *	13 ***	5	5	14	13 *
Regeneration	1	5 *	5	15 ***	0	0	10 **	7 **
Focal/multifocal necrosis	1	0	4	3	0	0	3	2
Diffuse centrilobular necrosis	1	3	4	2	1	0	0	2
Cytoplasmic vacuolization	3	0	6	8	4	6	9	9
Cystic degeneration	1	5 *	10 *	7 *	0	1	0	1

* Significantly different (P≤0.05) from the control group by logistic regression analysis
** P≤0.01
*** P≤0.001

Oral Cavity (Tongue or Pharynx): Squamous cell papilloma and squamous cell carcinoma of the oral cavity epithelium are uncommon neoplasms in untreated F344/N rats, with an average historical incidence in NTP 2-year studies less than 1% (range 0% to 2%; Tables A4d and B4d). In these studies, the incidence of squamous cell papilloma or carcinoma (combined) of the oral cavity was substantially increased in treated males and females (Table 18). In addition, hyperplasia of the epithelium of the palate was seen in two low-dose and four mid-dose female rats, and hyperplasia of the tongue was seen in one high-dose female rat. 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 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 (Plate 3). Fibroplasia and inflammation were sometimes seen with the invasion.

Small Intestine: A few adenocarcinomas of the small intestine occurred in male and female rats treated with C.I. Direct Blue 15, and a single adenomatous polyp was seen in one low-dose male rat (Table 19). These neoplasms occur rarely in untreated F344/N rats (mean <1%, range 0%-2%; Tables A4b and B4b). All of these neoplasms occurred in the high-dose groups except for the single adenomatous polyp in a low-dose male and one adenocarcinoma in a mid-dose female. One high-dose male had two adenocarcinomas of the small intestine. 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. Some adenocarcinomas contained large cystic spaces filled with mucus and debris (cystic mucinous adenocarcinoma). The adenomatous polyp was a pedunculated exophytic mass that consisted of a stalk-like core of fibrous tissue covered by numerous glandular structures lined by a single layer of well-differentiated columnar cells with abundant basophilic cytoplasm.

TABLE 18
Neoplasms of the Oral Cavity in F344/N Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Male				
Squamous Cell Papilloma[a]				
Overall rates[b]	0/50 (0%)	9/35 (26%)	18/65 (28%)	15/50 (30%)
Effective rates[c]	0/50 (0%)	9/34 (26%)	18/65 (28%)	15/47 (32%)
Terminal rates[d]	0/37 (0%)	3/8 (38%)	1/11 (9%)	0/2 (0%)
First incidence (days)	-[f]	316	460	372
Logistic regression tests[e]	P<0.001	P<0.001	P<0.001	P<0.001
Squamous Cell Carcinoma[g]				
Overall rates	1/50 (2%)	1/35 (3%)	6/65 (9%)	2/50 (4%)
Effective rates	1/50 (2%)	1/35 (3%)	6/65 (9%)	2/47 (4%)
Terminal rates	1/37 (3%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	680 (T)	293	502	479
Logistic regression tests	P=0.503	P=0.739N	P=0.141	P=0.461
Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rates	1/50 (2%)	10/35 (29%)	24/65 (37%)	17/50 (34%)
Effective rates	1/50 (2%)	10/35 (29%)	24/65 (37%)	17/47 (36%)
Terminal rates	1/37 (3%)	3/8 (38%)	1/11 (9%)	0/2 (0%)
First incidence (days)	680 (T)	293	460	372
Logistic regression tests	P<0.001	P=0.001	P<0.001	P<0.001
Female				
Squamous Cell Papilloma[h]				
Overall rates	2/50 (4%)	3/35 (9%)	12/65 (18%)	9/50 (18%)
Effective rates	2/49 (4%)	3/35 (9%)	12/63 (19%)	9/46 (20%)
Terminal rates	2/40 (5%)	0/13 (0%)	3/22 (14%)	1/4 (25%)
First incidence (days)	682 (T)	463	583	372
Logistic regression tests	P=0.015	P=0.491	P=0.008	P=0.035
Squamous Cell Carcinoma[i]				
Overall rates	0/50 (0%)	1/35 (3%)	8/65 (12%)	6/50 (12%)
Effective rates	0/49 (0%)	1/35 (3%)	8/64 (13%)	6/47 (13%)
Terminal rates	0/40 (0%)	1/13 (8%)	0/22 (0%)	1/4 (25%)
First incidence (days)	-	682 (T)	432	359
Logistic regression tests	P=0.015	P=0.277	P=0.023	P=0.023
Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rates	2/50 (4%)	4/35 (11%)	19/65 (29%)	15/50 (30%)
Effective rates	2/49 (4%)	4/35 (11%)	19/64 (30%)	15/47 (32%)
Terminal rates	2/40 (5%)	1/13 (8%)	3/22 (14%)	2/4 (50%)
First incidence (days)	682 (T)	463	432	359
Logistic regression tests	P<0.001	P=0.294	P<0.001	P=0.001

TABLE 18
Neoplasms of the Oral Cavity in F344/N Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15 (continued)

(T)Terminal kill
[a] Historical incidence for 2-year NTP studies of untreated control groups (mean ± standard deviation): 3/1596 (0.2% ± 0.6%)
[b] Number of tumor-bearing animals/number of animals necropsied
[c] Number of tumor-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 control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard tumors in animals dying prior to terminal kill as nonfatal. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[f] Not applicable; no tumors in animal group
[g] Historical incidence: 4/1596 (0.3% ± 0.7%)
[h] Historical incidence: 1/1643 (0.1% ± 0.4%)
[i] Historical incidence: 3/1643 (0.2% ± 0.6%)

TABLE 19
Neoplasms of the Small Intestine in F344/N Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Male				
Adenomatous Polyp				
Overall rates[a]	0/50 (0%)	1/35 (3%)	0/65 (0%)	0/50 (0%)
Adenocarcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	0/65 (0%)	2/50 (4%)
Effective rates[b]	0/47 (0%)	0/31 (0%)	0/59 (0%)	2/36 (6%)
Terminal rates[c]	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-[e]	-	-	479
Logistic regression tests[d]	P=0.078	-	-	P=0.304
Adenomatous Polyp or Adenocarcinoma[f]				
Overall rates	0/50 (0%)	1/35 (3%)	0/65 (0%)	2/50 (4%)
Effective rates	0/48 (0%)	1/33 (3%)	0/62 (0%)	2/42 (5%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	421	-	479
Logistic regression tests	P=0.309	P=0.573	-	P=0.304
Female				
Adenocarcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	1/65 (2%)	3/50 (6%)
Effective rates	0/49 (0%)	0/33 (0%)	1/56 (2%)	3/38 (8%)
Terminal rates	0/40 (0%)	0/13 (0%)	0/22 (0%)	0/4 (0%)
First incidence (days)	-	-	479	578
Logistic regression tests	P=0.032	-	P=0.688	P=0.075

[a] Number of tumor-bearing animals/number of animals necropsied
[b] Number of tumor-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 control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard tumors in animals dying prior to terminal kill as nonfatal.
[e] Not applicable; no tumors in animal group
[f] Historical incidence for 2-year NTP studies of untreated control groups (mean ± standard deviation): 5/1557 (0.3% ± 0.7%)

Large Intestine: Adenomatous polyps and adenocarcinomas of the large intestine mucosa also occur rarely in untreated F344/N rats (mean <1%, range 0% to 2%; Tables A4a and B4a). Several of these neoplasms occurred in treated male and female rats, but none were seen in untreated rats (Table 20). The incidences of adenomatous polyp, adenocarcinoma, and adenomatous polyp or adenocarcinoma (combined) were significantly increased in the high-dose male group. Adenomatous polyps of the large intestine had a histologic appearance similar to that of polyps occurring in the small intestine (Plate 4). Adenocarcinomas resembled polyps except that adenocarcinomas contained areas of invasion of the fibrous tissue core by neoplastic glandular epithelial cells.

Uterus: Uterine epithelial neoplasms occurred with somewhat higher incidence in treated females than in controls (Table 21). The incidence of adenoma or adenocarcinoma (combined) in the high-dose group was significantly increased and exceeded the range of historical control values for untreated female F344/N rats from NTP 2-year studies [4/1632 (0.2%), range 0% to 2%]. The incidence of endometrial stromal polyps from the 15-month interim evaluations and the 22-month studies combined was increased in the low- and mid-dose groups [control, 5/60 (8.3%); low-dose, 13/45 (28.9%); mid-dose, 14/65 (21.5%); high-dose, 7/60 (11.7%)]. Endometrial stromal polyps are commonly occurring neoplasms in untreated female F344/N rats, and all incidences in this study were well within the range of historical control values from NTP 2-year studies [341/1632 (21%), range 8% to 36%]. Because the incidence in the control group from this study is at the low end of the historical control range, the increased incidence of endometrial stromal polyps in the low- and mid-dose groups is not considered to be a treatment-related effect.

Brain: Malignant astrocytomas occurred in a few animals of each sex (males: control, 0/50; low-dose, 1/35; mid-dose, 1/65; high-dose, 2/50; females: control, 1/50; low-dose, 0/35; mid-dose, 2/65; high-dose, 1/50). Astrocytomas are uncommon neoplasms in untreated F344/N rats and are usually late-occurring neoplasms seen at necropsy. In these studies, there was substantial early mortality in treated groups, which greatly reduced the numbers of animals at risk for the occurrence of brain neoplasms. Consequently, malignant astrocytomas in treated animals may have been associated with the administration of C.I. Direct Blue 15.

Kidney: Two adenomas of renal tubule epithelium occurred in the high-dose male group. No primary renal neoplasms occurred in any of the other treated or control groups of either sex. The incidence of this neoplasm lies within the historical range for untreated male F344/N rats from 2-year NTP studies [10/1590 (1%), range 0% to 6%], and there was no treatment-related increase in the incidence of renal tubule epithelial hyperplasia, a lesion generally considered to be the precursor of renal tubule neoplasms. Hyperplasia was seen only in one low-dose and one high-dose male. Therefore, the occurrence of these two adenomas was not considered to be a treatment-related effect.

Adrenal Gland: The incidence of benign or malignant pheochromocytomas (combined) in the high-dose male group was significantly different from controls (control, 16/50; low-dose, 5/35; mid-dose 21/65; high-dose, 17/50). The incidence of adrenal medullary hyperplasia was similar across male dose groups (control, 4/50; low-dose, 5/35; mid-dose, 2/65; high-dose, 5/50). The overall incidences of hyperplasia and pheochromocytoma were essentially identical in the control and high-dose groups, but there was marked early mortality in the high-dose group and the first occurrence of a pheochromocytoma was during week 65 of the study in the high-dose group and during week 91 in the control group. Because the statistical analysis results reflect survival data as well as numbers of neoplasms, and because the neoplasms occurred earlier in high-dose males than in control males, there is a statistically significant difference between the control and high-dose groups. Pheochromocytomas are relatively slow-growing neoplasms that are seldom seen before one year of age. They begin as focal noncompressive proliferative lesions of the adrenal medullary cells, diagnosed as hyperplasias. As the proliferative lesions increase in size, they displace and compress the normal parenchyma, at which point they are diagnosed as pheochromocytomas. The neoplasms continue to grow and can reach a centimeter

TABLE 20
Neoplasms of the Large Intestine in F344/N Rats in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Male				
Adenomatous Polyp				
Overall rates[a]	0/50 (0%)	1/35 (3%)	2/65 (3%)	5/50 (10%)
Effective rates[b]	0/45 (0%)	1/31 (3%)	2/59 (3%)	5/33 (15%)
Terminal rates[c]	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	–[e]	579	559	502
Logistic regression tests[d]	P=0.005	P=0.471	P=0.317	P=0.010
Adenocarcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	4/65 (6%)	3/50 (6%)
Effective rates	0/45 (0%)	0/31 (0%)	4/58 (7%)	3/32 (9%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	–	–	628	505
Logistic regression tests	P=0.034	–	P=0.072	P=0.156
Adenomatous Polyp or Adenocarcinoma[f]				
Overall rates	0/50 (0%)	1/35 (3%)	6/65 (9%)	8/50 (16%)
Effective rates	0/45 (0%)	1/31 (3%)	6/59 (10%)	8/33 (24%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	–	579	559	502
Logistic regression tests	P<0.001	P=0.471	P=0.030	P=0.002
Female				
Adenomatous Polyp				
Overall rates	0/50 (0%)	0/35 (0%)	3/65 (5%)	1/50 (2%)
Effective rates	0/45 (0%)	0/18 (0%)	3/35 (9%)	1/9 (11%)
Terminal rates	0/40 (0%)	0/13 (0%)	2/22 (9%)	0/4 (0%)
First incidence (days)	–	–	640	646
Logistic regression tests	P=0.062	–	P=0.094	P=0.347

[a] Number of tumor-bearing animals/number of animals necropsied
[b] Number of tumor-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 control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard tumors in animals dying prior to terminal kill as nonfatal.
[e] Not applicable; no tumors in animal group
[f] Historical incidence for 2-year NTP studies of untreated control groups (mean ± standard deviation): 2/1541 (0.1% ± 0.5%)

TABLE 21
Neoplasms of the Uterus in Female F344/N Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Adenocarcinoma				
Overall rates[a]	1/50 (2%)	0/35 (0%)	0/65 (0%)	3/50 (6%)
Effective rates[b]	1/46 (2%)	0/21 (0%)	0/45 (0%)	3/17 (18%)
Terminal rates[c]	1/40 (3%)	0/13 (0%)	0/22 (0%)	0/4 (0%)
First incidence (days)	682 (T)	-[e]	-	607
Logistic regression tests[d]	P=0.042	P=0.723N	P=0.619N	P=0.132
Adenoma or Adenocarcinoma[f]				
Overall rates	1/50 (2%)	0/35 (0%)	1/65 (2%)	4/50 (8%)
Effective rates	1/46 (2%)	0/21 (0%)	1/45 (2%)	4/17 (24%)
Terminal rates	1/40 (3%)	0/13 (0%)	1/22 (5%)	1/4 (25%)
First incidence (days)	682 (T)	-	682 (T)	607
Logistic regression tests	P=0.004	P=0.723N	P=0.623	P=0.025

(T)Terminal kill
[a] Number of tumor-bearing animals/number of animals necropsied
[b] Number of tumor-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 control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard tumors in animals dying prior to terminal kill as nonfatal. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no tumors in animal group
[f] Historical incidence for 2-year NTP studies of untreated control groups (mean ± standard deviation): 4/1632 (0.2% ± 0.7%)

or more in diameter. Thus, pheochromocytomas range in size from minute lesions which can only be diagnosed microscopically to large masses which can replace the entire adrenal parenchyma. Pheochromocytomas occur commonly in aged male F344/N rats and are usually considered incidental findings. The apparent earlier onset of pheochromocytomas in high-dose males in this study is presumably due to the high early mortality in this group, which resulted in a larger number of high-dose animals being examined at an earlier age than was the case for controls. It is likely that some of the control animals may have had pheochromocytomas by ages similar to those of the high-dose males, but they were not detected in control animals until later in life because the control animals lived longer. No difference in the incidence of hyperplasia, the precursor of pheochromocytoma, was found between the control and high-dose groups. Consequently, the statistically significant difference between the control and high-dose male groups is not considered to represent a treatment-related effect.

Spleen: Hematopoietic cell proliferation occurred with increased incidence in the spleens of treated male and female rats (males: control, 1/50; low-dose, 3/35; mid-dose, 10/64; high-dose, 17/50; females: control, 5/50; low-dose, 12/35; mid-dose, 20/65; high-dose, 18/50). This effect was considered to be secondary to the inflammation associated with the neoplasms in treated animals.

Bone Marrow: The incidence of hyperplasia of the bone marrow was markedly increased in treated male rats (control, 1/49; low-dose, 14/35; mid-dose, 20/63; high-dose, 16/50). The hyperplasia was due to an increase in hematopoietic cell proliferation secondary to neoplasm-associated inflammation in treated animals.

Heart: The incidence of thrombus within the atrium of the heart was higher in the mid- and high-dose male groups than in the controls (control, 2/50; low-dose, 3/35; mid-dose, 17/65; high-dose, 12/50).

GENETIC TOXICITY

C.I. Direct Blue 15 was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 when tested in a standard preincubation protocol at concentrations of 100 to 10,000 μg/plate in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table C1) (Mortelmans *et al.*, 1986). As do most benzidine congener dyes, this compound requires reductive metabolism of the azo bonds to release the parent amine, which can then be oxidatively metabolized to an active mutagen. When tested in such a reductive metabolism protocol, C.I. Direct Blue 15 was mutagenic in *S. typhimurium* strain TA1538 (Table C2) (Reid *et al.*, 1984a,b). Some mutagenic activity was observed in the presence of rat S9 without prior reduction, but the mutagenicity was greatly increased following reduction. The fact that the mutagenic activity of C.I. Direct Blue 15 was less than expected in the bacterial reduction system, based on the comparative activity of the dimethoxybenzidine control, can be explained by the small proportion of dye that was reduced using this system. In a test system using a flavin mononucleotide reduction and hamster S9 activation protocol, the mutagenic activity of C.I. Direct Blue 15 was greater than expected. This increase in mutagenic activity may have resulted from the formation of additional reduction products in the crude dye mixture that was tested.

In cytogenetic tests with Chinese hamster ovary cells, C.I. Direct Blue 15 did not induce sister chromatid exchanges when tested at concentrations up to 750 μg/ml in the absence of S9, or at concentrations up to 2,500 μg/ml in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Table C3) (Galloway *et al.*, 1987). No induction of chromosomal aberrations was observed in Chinese hamster ovary cells treated with up to 2,250 μg/ml C.I. Direct Blue 15 without S9 or 2,500 μg/ml with S9 (Table C4) (Galloway *et al.*, 1987). Reductive metabolism was not used in these cytogenetic tests.

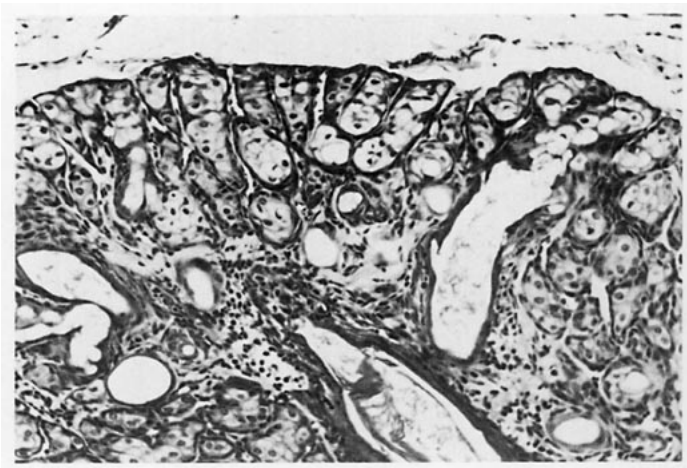


PLATE 1
Sebaceous gland adenoma of the skin in an F344/N rat administered 2,500 ppm C.I. Direct Blue 15 in drinking water for two years. Magnification 150×

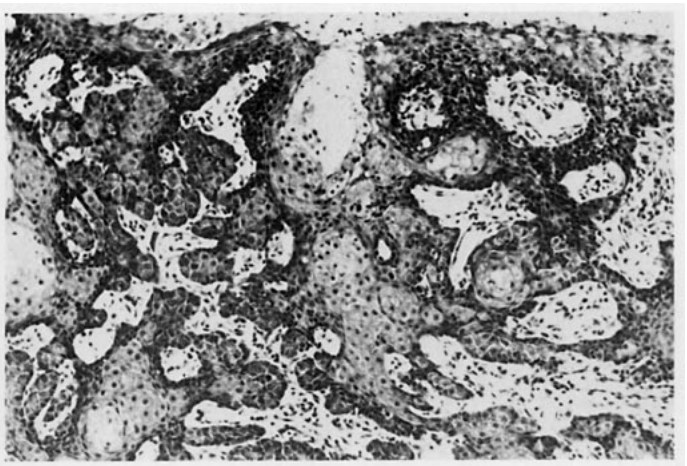


PLATE 2
Preputial gland carcinoma in an F344/N rat administered 1,250 ppm C.I. Direct Blue 15 in drinking water for two years. Magnification 150×

PLATE 3
Squamous cell carcinoma of the tongue in an F344/N rat administered 2,500 ppm C.I. Direct Blue 15 in drinking water for two years. Magnification 60×

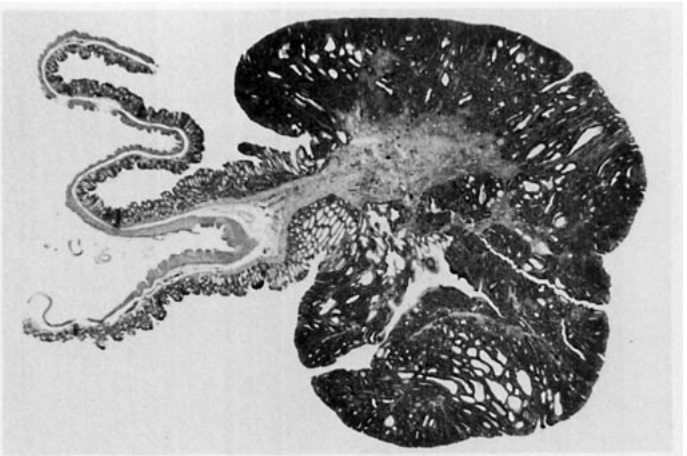


PLATE 4
Adenomatous polyp of the large intestine in an F344/N rat administered 2,500 ppm C.I. Direct Blue 15 in drinking water for two years. Magnification 10×

DISCUSSION AND CONCLUSIONS

The NTP's Benzidine Dye Initiative is a program that was developed to study the toxicology and carcinogenicity of the class of dyes derived from benzidine, 3,3'-dimethylbenzidine dihydrochloride, and 3,3'-dimethoxybenzidine dihydrochloride. The dyes selected for study were representative of the more than 90 benzidine dyes in use. These studies examined the toxic and carcinogenic properties of C.I. Direct Blue 15 (desalted industrial grade). The dye examined was the product to which workers are potentially exposed. The purity of the dye was determined to be about 50%, with approximately 35 impurities.

14-Day Studies

Groups of five rats of each sex were administered C.I. Direct Blue 15 in drinking water at dose levels of 0, 1,250, 2,500, 5,000, 10,000 or 30,000 ppm for 14 days. All control and treated rats survived. Final mean body weights of rats in the 30,000 ppm groups were lower than controls (males, 8%; females, 34%). Water consumption declined with increased dose. Clinical findings included blue-stained eyes, skin, and feces in all treated animals. Organs and tissues of treated rats were also stained blue. Animals receiving 30,000 ppm showed histologic evidence of mild hepatotoxicity; females at this dose level also showed evidence of nephrotoxicity and thymic lymphoid depletion.

13-Week Studies

Male rats received C. I. Direct Blue 15 in drinking water at doses of 0, 1,250, 2,500, 5,000, 10,000, or 30,000 ppm, and female rats received doses of 0, 630, 1,250, 2,500, 5,000, or 10,000 ppm. Seven of ten male rats receiving 30,000 ppm died during the treatment period; animals in all other groups survived until the end of the studies. Final mean body weights were 8% and 31% lower than that of controls in male rats given 10,000 and 30,000 ppm and were 3% and 6% lower than that of controls in female rats given 5,000 and 10,000 ppm. As in the 14-day studies, major organs and tissues from hepatic toxicity was observed in the seven males that died before the end of the studies. An increase in the severity of nephropathy was seen in both males and females receiving 10,000 ppm, and relative kidney weight was higher in rats that received 5,000 ppm or greater than in controls. Because of this kidney toxicity, a high dose of 2,500 ppm was selected for the 22-month studies.

22-Month Studies

The toxicity and carcinogenicity studies of C.I. Direct Blue 15 were designed to last 2 years, but were terminated at 22 months because of extensive early deaths in the treated groups. The allotment of animals to groups was based on study designs recommended by Portier and Hoel (1984): at study initiation, 70 animals per sex received 0 ppm or 2,500 ppm, 45 animals per sex received 630 ppm, and 75 animals per sex received 1,250 ppm. The amount of compound consumed per day by rats at the three dose levels was approximately 45, 90, or 215 mg/kg for male rats and 50, 100, or 200 mg/kg for female rats. Compound consumption calculations were based on average water consumption by groups of animals during these studies. Ten animals in the control and 2,500 ppm groups were evaluated at 9 months and 10 animals from each dose group were evaluated at 15 months. At week 97, the final mean body weights relative to controls of the 630, 1,250, and 2,500 ppm groups were 95%, 91%, and 81% for male rats and 91% for all three dosed female rat groups.

At the 9-month interim evaluation, a Zymbal's gland adenoma was seen in one high-dose male rat, and clitoral gland carcinomas were seen in three high-dose female rats. At the 15-month interim evaluation, two low-dose females, one mid-dose female, three high-dose males, and three high-dose females had Zymbal's gland neoplasms; two mid-dose female rats and one high-dose female rat had clitoral gland carcinomas. At the 9-month and 15-month interim evaluations, lesions were also noted in the skin, preputial gland, intestines, liver, and oral cavity of treated animals, and the percentages of animals with these lesions were higher at 15 months.

In the 22-month studies, chemical-related neoplasms and nonneoplastic lesions were found at many sites, including the Zymbal's gland, skin, oral cavity, intestine, liver, and preputial and clitoral glands in male and female rats; these findings are similar to those observed in the 15-month 3,3'-dimethylbenzidine dihydrochloride and the 21-month 3,3'-dimethoxybenzidine dihydrochloride studies. In the NTP database of over 350 long-term rodent studies, 18 studies include the Zymbal's gland as a site for neoplasm formation in the rat and 16 chemicals caused neoplasms in the skin in the rat; 12 chemicals caused neoplasms in both the Zymbal's gland and skin (Table 22). Many of these chemicals have in common a structure that contains a nitrogen-aromatic bond. Most chemicals that caused Zymbal's gland lesions or skin lesions also caused lesions at other sites in the body and were positive in the NTP *Salmonella typhimurium* mutagenicity assays, as was the case for C.I. Direct Blue 15, 3,3'-dimethoxybenzidine dihydrochloride, and 3,3'-dimethylbenzidine dihydrochloride. The incidences of neoplasms of the epidermis of the skin, the oral cavity epithelium, and the epithelium of the Zymbal's, clitoral, and preputial glands were high in these studies, and neoplasms often occurred at more than one of these sites in the same animal.

The incidences of Zymbal's gland carcinoma or adenoma were markedly increased in rats receiving C.I. Direct Blue 15. The incidences in the treated groups were well above the historical mean for Zymbal's gland lesions in rats in the NTP database for 2-year rodent studies even though the historical rates listed in this report are for 2-year rodent studies, whereas the C.I. Direct Blue 15 study was for 22 months.

The incidences of skin squamous cell neoplasms in male rats (2/50, 4/35, 11/65, 19/50) and in female rats (0/50, 2/35, 6/65, 5/50) were increased above the NTP historical mean for untreated controls (males, 1.8%; females, 0.4%). The incidences of skin basal cell neoplasms were significantly increased in male rats (2/50, 9/35, 27/65, 28/50), but not in female rats (1/50, 0/35, 1/65, 0/50). Exposure of the skin to C.I. Direct Blue 15 may have occurred through the systemic distribution of the chemical or through direct contact with the skin while the animals were grooming.

In female rats, the incidence of clitoral gland adenomas or carcinomas was markedly increased in the treated groups (7/50, 11/31, 24/64, 27/50), and in males the incidence of preputial gland neoplasms was increased in the mid-dose group (8/49, 5/35, 23/64, 9/48). The increase in the incidence of liver neoplasms was more marked in male rats (0/50, 6/35, 9/65, 11/50) than in female rats (0/50, 0/35, 2/65, 5/50), although the incidences of liver neoplasms in both sexes of treated animals were above the mean incidences in the NTP historical database (males, 4.9%; females, 2.3%).

Oral cavity and intestinal neoplasms were seen in male and female rats; these sites may also have been exposed to the chemical either through direct contact or through the systemic circulation. The incidences of squamous cell neoplasms in the oral cavity were significantly increased in male rats (1/50, 10/35, 24/65, 17/50) and female rats (2/50, 4/35, 19/65, 15/50). The incidences of large intestine neoplasms (males: 0/50, 1/35, 6/65, 8/50; females: 0/50, 0/35, 3/65, 1/50) and small intestine neoplasms (males: 0/50, 1/35, 0/65, 2/50; females: 0/50, 0/35, 1/65, 3/50) were increased in male and female rats at 22 months; neoplasms of the large and small intestine were also seen in a few dosed animals evaluated at 15 months. The NTP historical mean for 2-year studies for oral cavity and intestinal neoplasms in rats ranges from 0% to 0.3%.

The incidence of adenoma or adenocarcinoma of the uterus was significantly increased in high-dose females (1/50, 0/35, 1/65, 4/50). These neoplasms occur rarely in untreated rats; the historical mean for NTP 2-year studies is 0.3%.

Neoplasms were found in the brain of males (0/50, 1/35, 1/65, 2/50) and females (1/50, 0/35, 2/65, 1/50). The brain neoplasms were malignant astrocytomas, an uncommon malignant neoplasm of glial cell origin. The incidences of these neoplasms were only marginally increased. However, in view of the reduced survival of treated rats and the low spontaneous occurrence of malignant astrocytomas (historical incidence: males, 0.63%; females, 0.92%), these neoplasms may have been related to chemical exposure. A low incidence of brain astrocytomas was also seen in the rat studies of 3,3'-dimethoxybenzidine dihydrochloride and 3,3'-dimethylbenzidine dihydrochloride.

TABLE 22
Evidence of Zymbal's Gland and Skin Neoplasms in Rats and *Salmonella* Mutagenicity for Selected National Toxicology Program Chemicals

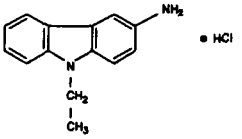
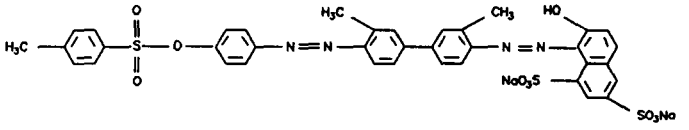
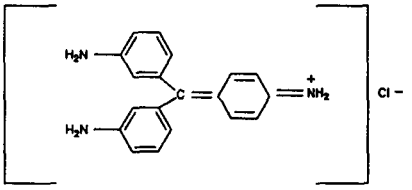
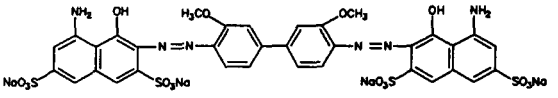
Chemical Name/ Structure	Technical Report Number	Zymbal's Gland Neoplasms M	Zymbal's Gland Neoplasms F	Skin Neoplasms M	Skin Neoplasms F	*Salmonella* Mutagenicity Results
3-Amino-9-Ethylcarbazole HCl 	93	+	+	+		+
Benzene 	289	+	+	+		
C.I. Acid Red 114 	405	+	+	+	+	+
C.I. Basic Red 9 Monohydrochloride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

TABLE 22
Evidence of Zymbal's Gland and Skin Neoplasms in Rats and *Salmonella* Mutagenicity for Selected National Toxicology Program Chemicals (continued)

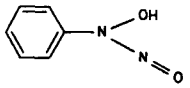
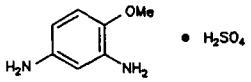
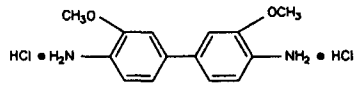
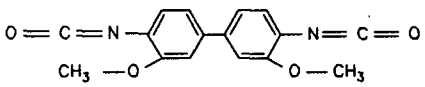
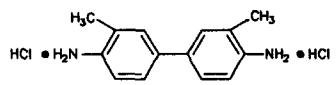
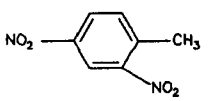
Chemical Name/ Structure	Technical Report Number	Zymbal's Gland Neoplasms M	Zymbal's Gland Neoplasms F	Skin Neoplasms M	Skin Neoplasms F	*Salmonella* Mutagenicity Results
Cupferron	100	+				+
						
2,4-Diaminoanisole Sulfate	84	+	+	+		+
						
3,3′-Dimethoxybenzidine Dihydrochloride	372	+	+	+	+	+
	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

TABLE 22
Evidence of Zymbal's Gland and Skin Neoplasms in Rats and *Salmonella* Mutagenicity for Selected National Toxicology Program Chemicals (continued)

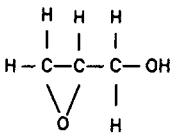
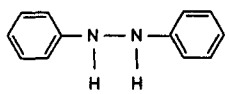
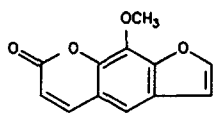
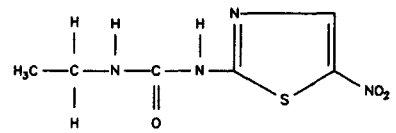
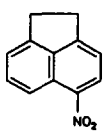
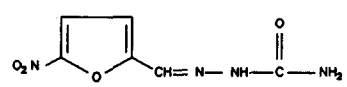
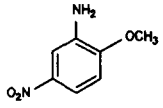
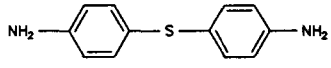
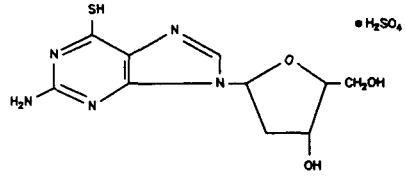
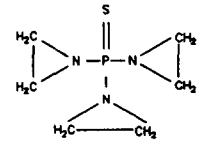
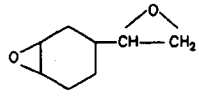
Chemical Name/ Structure	Technical Report Number	Zymbal's Gland Neoplasms M	Zymbal's Gland Neoplasms F	Skin Neoplasms M	Skin Neoplasms F	*Salmonella* Mutagenicity Results
Glycidol 	374	+		+		+
Hydrazobenzene 	92	+				+
8-Methoxypsoralen 	359	+				+
Nithiazide 	146			+		+
5-Nitroacenaphthene 	118	+	+			+
Nitrofurazone 	337			+		+

TABLE 22
Evidence of Zymbal's Gland and Skin Neoplasms in Rats and *Salmonella* Mutagenicity for Selected National Toxicology Program Chemicals (continued)

Chemical Name/ Structure	Technical Report Number	Zymbal's Gland Neoplasms M F	Skin Neoplasms M F	*Salmonella* Mutagenicity Results
5-Nitro-*o*-Anisidine	127	+ +	+	+
				
4,4′-Thiodianiline	47	+ +		+
				
β-Thioguanidine Deoxyriboside	57	+		
				
Tris(Aziridinyl)Phosphine Sulfide	58	+ +	+ +	+
				
4-Vinyl-1-Cyclohexene Diepoxide	362		+ +	+
				

When analyzed by life table or logistic regression analyses, the incidence of mononuclear cell leukemia was significantly increased in female rats (7/50, 13/35, 27/65, 15/50) and marginally increased in male rats (17/50, 19/35, 28/65, 20/50). A marginal increase in the incidence of mononuclear cell leukemia was also seen in female rats in the studies of 3,3′-dimethylbenzidine. The historical mean for mononuclear cell leukemia in female rats is 13.9%.

The benzidine dyes have been found to be genotoxic agents (Table 23). Available data from metabolism studies indicate that C.I. Direct Blue 15 is metabolized to its parent compound (dimethoxybenzidine), the likely precursor of the active moiety. C.I. Direct Blue 15 was negative in the standard NTP *Salmonella* assays, but was positive in *Salmonella typhimurium* strain TA1538 when reductive metabolism was used. C.I. Direct Blue 15 has aromatic-amine groupings, which are considered to be "structural alerts" for genotoxic activity (Ashby and Tennant, 1988).

Tumor development and formation may occur through several mechanisms. Talaska *et al.* (1987) have shown that benzidine or a benzidine metabolite can form DNA adducts in the mouse liver and have suggested that benzidine may cause tumor formation through somatic mutations, which allow a cell to escape the growth control of the organism and become a neoplasm. Büsser and Lutz (1987) investigated the stimulation of liver DNA synthesis and cell proliferation by DNA-binding carcinogens (benzidine, carbon tetrachloride, and aflatoxin) and hepatic tumor promoters (DDT, phenobarbital, and thioacetamide) and found that the DNA-binding carcinogens did not stimulate liver DNA synthesis, but the tumor promoters did. Similar studies have not been carried out with C.I. Direct Blue 15.

TABLE 23
Comparison of National Toxicology Program Mutagenicity Test Results for Selected Benzidine Dyes[a]

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

[a] Results are presented as result of test without S9/result of test with S9. 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.

[b] Positive in *Salmonella* 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. These compounds are not mutagenic in TA1538 in the absence of a reducing system (Reid *et al.*, 1984a).

[c] Not mutagenic with reductive metabolism.

In other studies, BALB/c mice were treated with 3,3′-dimethylbenzidine dihydrochloride in drinking water at doses ranging from 5 to 150 ppm for up to 116 weeks, or with 3,3′-dimethoxybenzidine dihydrochloride in the drinking water at doses ranging from 20 to 630 ppm for up to 112 weeks. Dose-related increases in the incidence of lung alveolar cell neoplasms were seen in males, but not in females, after 3,3′-dimethylbenzidine dihydrochloride treatment. No increases in neoplasm incidence were seen in the mice treated with 3,3′-dimethoxybenzidine dihydrochloride (Schieferstein *et al.*, 1990). The spectrum of lesions observed in these mouse studies was quite different from that seen in the NTP rat studies.

Benzidine and related aromatic amines produce neoplasms in a wide variety of tissues in experimental animals. In humans, exposure to benzidine is associated with cancer of the urinary bladder (Zavon *et al.*, 1973). In mice, the liver is the major organ affected (Bonser *et al.*, 1956; Vesselinovitch *et al.*, 1975; Littlefield *et al.*, 1983; IARC, 1987), and in rats, benzidine and other aminobiphenyls cause neoplasms in the Zymbal's gland, mammary gland, skin, intestine, and liver. These differences may be related to species-specific and organ-specific differences in metabolism.

A number of aromatic amines cause neoplasms in the Zymbal's gland. Reported to be deficient in sulfotransferase activity (Irving *et al.*, 1971) and transacylase activity (Bartsch *et al.*, 1973), the Zymbal's gland is capable of hydroxylating compounds via cytochrome P_{450}-dependent enzymatic pathways (Pohl and Fouts, 1983). Susceptibility of a species to the carcinogenic action of aromatic amines depends 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 additional activation to form reactive esters, which act as ultimate carcinogens (Miller and Miller, 1977). Formation of different esters by different species may result in variations in organ specificity (Cohen, 1983).

Neoplasm Transplant Study

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.

Oncogene Activation

Neoplasms obtained from control rats and rats exposed to 3,3′-dimethoxybenzidine dihydrochloride or C.I. Direct Blue 15 (a 3,3′-dimethoxybenzidine-derived dye) were assayed for the presence of activated protooncogenes by the NIH 3T3 DNA transfection assay (Reynolds *et al.*, 1990). Oncogenes detectable by DNA transfection analysis were present in 21/27 skin, clitoral gland, or preputial gland neoplasms that had been induced by 3,3′-dimethoxybenzidine dihydrochloride or C.I. Direct Blue 15. DNA from either benign or malignant neoplasms was capable of inducing morphologically transformed foci in NIH 3T3 mouse fibroblast cultures.

Thirteen of the chemical-induced neoplasm types were of epidermal origin and were classified as basal or squamous cell neoplasms of the skin; activated *ras* oncogenes were detected at a high frequency in

these neoplasms (11/13). Histogenetically related neoplasms of the clitoral and preputial glands also had a high frequency of activated *ras* oncogenes (10/14). In contrast, the occurrence of *ras* oncogene activation in spontaneous epithelial neoplasms of the F344/N rat is low (1/21).

It is possible that chemical-induced neoplasms were derived from a common epidermal progenitor stem-cell population that was susceptible to electrophilic attack by activated metabolites of 3,3′-dimethoxybenzidine dihydrochloride or C.I. Direct Blue 15. A relatively high percentage (62%) of the chemical-induced rat neoplasms contained activated alleles of either H-*ras* or N-*ras*.

CONCLUSIONS

Under the conditions of these 22-month drinking water studies, there was *clear evidence of carcinogenic activity** of C.I. Direct Blue 15 (desalted industrial grade) in male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity, and small and large intestine. Increased incidences of mononuclear cell leukemia and neoplasms of the brain may have been related to chemical administration. There was *clear evidence of carcinogenic activity* of C.I. Direct Blue 15 in female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity, small and large intestine, and uterus, and by mononuclear cell leukemia.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of peer review comments and the public discussion on this Technical Report appear on page 11.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 22-MONTH DRINKING WATER STUDY OF C.I. DIRECT BLUE 15

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
9-Month interim evaluation	10	0	0	10
15-Month interim evaluation	10	10	10	10
Early deaths				
Natural deaths	5	12	24	12
Moribund kills	8	15	30	34
Accidental deaths				2
Survivors				
Terminal sacrifice	37	8	11	2
Animals examined microscopically	50	35	65	50
Alimentary System				
Intestine large, cecum	(50)	(35)	(61)	(50)
Intestine large, colon	(50)	(35)	(62)	(50)
Adenocarcinoma			4 (6%)	3 (6%)
Polyp adenomatous			1 (2%)	1 (2%)
Ascending colon, polyp adenomatous		1 (3%)		
Descending colon, polyp adenomatous			1 (2%)	4 (8%)
Intestine small, duodenum	(49)	(35)	(60)	(50)
Adenocarcinoma, cystic, mucinous				1 (2%)
Intestine small, ileum	(49)	(35)	(60)	(50)
Intestine small, jejunum	(49)	(35)	(60)	(50)
Adenocarcinoma				1 (2%)
Adenocarcinoma, cystic, mucinous				1 (2%)
Carcinoma, metastatic		1 (3%)		
Polyp adenomatous		1 (3%)		
Liver	(50)	(35)	(65)	(50)
Carcinoma, metastatic		1 (3%)		
Hepatocellular carcinoma			1 (2%)	4 (8%)
Neoplastic nodule		5 (14%)	7 (11%)	4 (8%)
Neoplastic nodule, multiple		1 (3%)	1 (2%)	3 (6%)
Mesentery	(8)	(6)	(11)	(6)
Carcinoma, metastatic		1 (17%)		
Sarcoma			1 (9%)	
Pancreas	(49)	(35)	(65)	(50)
Pharynx	(1)	(9)	(23)	(20)
Squamous cell carcinoma			1 (4%)	
Palate, papilloma squamous		9 (100%)	16 (70%)	15 (75%)
Palate, papilloma squamous, multiple			1 (4%)	
Palate, squamous cell carcinoma	1 (100%)		3 (13%)	2 (10%)
Salivary glands	(50)	(35)	(65)	(50)
Schwannoma malignant			2 (3%)	
Bilateral, schwannoma malignant			1 (2%)	
Stomach, forestomach	(49)	(35)	(65)	(50)
Carcinoma, metastatic		1 (3%)		
Papilloma squamous			1 (2%)	
Stomach, glandular	(49)	(34)	(65)	(50)
Tongue		(2)	(4)	(2)
Papilloma squamous			1 (25%)	2 (100%)
Squamous cell carcinoma		1 (50%)	2 (50%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Cardiovascular System				
Heart	(50)	(35)	(65)	(50)
Carcinoma, metastatic, Zymbal's gland				1 (2%)
Schwannoma malignant				1 (2%)
Endocrine System				
Adrenal gland, cortex	(50)	(35)	(65)	(50)
Adrenal gland, medulla	(50)	(35)	(65)	(50)
Pheochromocytoma malignant			2 (3%)	1 (2%)
Pheochromocytoma complex				1 (2%)
Pheochromocytoma benign	12 (24%)	3 (9%)	10 (15%)	14 (28%)
Bilateral, pheochromocytoma benign	4 (8%)	2 (6%)	9 (14%)	3 (6%)
Islets, pancreatic	(50)	(35)	(65)	(50)
Adenoma	1 (2%)		1 (2%)	
Pituitary gland	(49)	(34)	(63)	(50)
Pars distalis, adenoma	5 (10%)	1 (3%)	3 (5%)	3 (6%)
Thyroid gland	(50)	(35)	(65)	(50)
Bilateral, C-cell, adenoma			1 (2%)	
Bilateral, C-cell, carcinoma	1 (2%)		1 (2%)	
C-cell, adenoma	5 (10%)	8 (23%)	6 (9%)	4 (8%)
C-cell, carcinoma	2 (4%)	1 (3%)	1 (2%)	1 (2%)
Follicular cell, adenoma		4 (11%)	1 (2%)	
Follicular cell, carcinoma			3 (5%)	
General Body System				
Tissue NOS		(1)		
Carcinoma		1 (100%)		
Genital System				
Epididymis	(50)	(35)	(64)	(50)
Carcinoma, metastatic, multiple		1 (3%)		
Preputial gland	(49)	(35)	(64)	(48)
Adenoma	5 (10%)	1 (3%)	10 (16%)	8 (17%)
Carcinoma	2 (4%)	2 (6%)	10 (16%)	1 (2%)
Bilateral, adenoma	1 (2%)	1 (3%)	2 (3%)	
Bilateral, carcinoma		1 (3%)	1 (2%)	
Prostate	(50)	(35)	(64)	(49)
Adenoma			1 (2%)	
Schwannoma malignant			1 (2%)	
Seminal vesicle	(47)	(32)	(57)	(42)
Carcinoma, metastatic		1 (3%)		
Schwannoma malignant, metastatic, prostate			1 (2%)	
Testes	(50)	(35)	(65)	(50)
Carcinoma, metastatic		1 (3%)		
Bilateral, interstitial cell, adenoma	43 (86%)	29 (83%)	52 (80%)	33 (66%)
Interstitial cell, adenoma	5 (10%)	3 (9%)	9 (14%)	10 (20%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Hematopoietic System				
Bone marrow	(49)	(35)	(63)	(50)
Lymph node	(50)	(35)	(64)	(50)
Axillary, carcinoma, metastatic				1 (2%)
Axillary, squamous cell carcinoma, metastatic, skin				1 (2%)
Mediastinal, squamous cell carcinoma, metastatic				1 (2%)
Lymph node, mandibular	(50)	(35)	(62)	(50)
Squamous cell carcinoma, metastatic, tongue			1 (2%)	
Mediastinal, carcinoma, metastatic, multiple, Zymbal's gland				1 (2%)
Lymph node, mesenteric	(50)	(35)	(64)	(50)
Carcinoma, metastatic		1 (3%)		
Spleen	(50)	(35)	(64)	(50)
Carcinoma, metastatic		1 (3%)		
Thymus	(43)	(34)	(51)	(43)
Carcinoma, metastatic, Zymbal's gland				1 (2%)
Integumentary System				
Mammary gland	(48)	(34)	(59)	(42)
Fibroadenoma			1 (2%)	
Skin	(50)	(35)	(65)	(50)
Basal cell adenoma	2 (4%)	5 (14%)	15 (23%)	9 (18%)
Basal cell adenoma, multiple		3 (9%)	8 (12%)	17 (34%)
Basal cell carcinoma		2 (6%)	3 (5%)	10 (20%)
Basal cell carcinoma, multiple			1 (2%)	
Carcinoma			1 (2%)	
Keratoacanthoma	2 (4%)	1 (3%)	7 (11%)	2 (4%)
Papilloma squamous	2 (4%)	3 (9%)	3 (5%)	7 (14%)
Papilloma squamous, multiple			2 (3%)	1 (2%)
Squamous cell carcinoma		1 (3%)	7 (11%)	7 (14%)
Squamous cell carcinoma, multiple				6 (12%)
Sebaceous gland, adenoma		1 (3%)	6 (9%)	3 (6%)
Sebaceous gland, adenoma, multiple			1 (2%)	
Subcutaneous tissue, carcinoma, metastatic, Zymbal's gland				1 (2%)
Subcutaneous tissue, fibroma	2 (4%)	2 (6%)	5 (8%)	3 (6%)
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Subcutaneous tissue, neurofibrosarcoma			1 (2%)	
Subcutaneous tissue, sarcoma		1 (3%)		1 (2%)
Musculoskeletal System				
Bone	(1)	(1)	(1)	
Cranium, carcinoma, metastatic, Zymbal's gland			1 (100%)	
Vertebra, osteoma		1 (100%)		
Skeletal muscle				(1)
Back, schwannoma malignant, metastatic, spinal cord				1 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Nervous System				
Brain	(50)	(35)	(65)	(50)
Cerebrum, astrocytoma malignant		1 (3%)	1 (2%)	1 (2%)
Medulla, astrocytoma malignant				1 (2%)
Spinal cord				(1)
Nerve, schwannoma malignant				1 (100%)
Respiratory System				
Lung	(50)	(35)	(65)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (3%)	4 (6%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Carcinoma, metastatic		1 (3%)		
Carcinoma, metastatic, thyroid gland		1 (3%)		
Carcinoma, metastatic, Zymbal's gland			1 (2%)	
Carcinoma, metastatic, multiple, Zymbal's gland		1 (3%)	1 (2%)	1 (2%)
Neoplasm NOS, metastatic, uncertain primary site			1 (2%)	
Neurofibrosarcoma, metastatic, multiple, skin			1 (2%)	
Squamous cell carcinoma, metastatic				1 (2%)
Squamous cell carcinoma, metastatic, skin				1 (2%)
Squamous cell carcinoma, metastatic, multiple, skin			1 (2%)	2 (4%)
Nose	(50)	(35)	(65)	(49)
Squamous cell carcinoma		1 (3%)	1 (2%)	
Special Senses System				
Zymbal's gland	(50)	(35)	(64)	(50)
Adenoma		2 (6%)	2 (3%)	4 (8%)
Carcinoma	1 (2%)	3 (9%)	8 (13%)	17 (34%)
Urinary System				
Kidney	(50)	(35)	(65)	(50)
Carcinoma, metastatic		1 (3%)		
Renal tubule, adenoma				2 (4%)
Urinary bladder	(50)	(35)	(65)	(50)
Carcinoma, metastatic		1 (3%)		
Schwannoma malignant, metastatic, prostate			1 (2%)	
Systemic Lesions				
Multiple organs[a]	(50)	(35)	(65)	(50)
Leukemia mononuclear	17 (34%)	19 (54%)	28 (43%)	20 (40%)
Mesothelioma malignant	1 (2%)	3 (9%)	2 (3%)	2 (4%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Tumor Summary				
Total animals with primary neoplasms[b]	48	34	65	48
Total primary neoplasms	116	125	276	236
Total animals with benign neoplasms	48	33	62	46
Total benign neoplasms	91	88	188	153
Total animals with malignant neoplasms	23	27	54	45
Total malignant neoplasms	25	37	88	83
Total animals with secondary neoplasms[c]	1	5	8	9
Total secondary neoplasms	2	26	12	15
Total animals with malignant neoplasms of uncertain primary site			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 A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm

Number of Days on Study	3 3 3	3 5 7	4 4 5	4 9 5	5 0 1	5 5 8	5 6 5	5 8 9	6 3 7	6 4 2	6 5 9	6 5 9	6 6 3	6 8 0	6 8 0	6 8 0	6 8 0	6 8 0	6 8 0	6 8 0	6 8 0	6 8 1	6 8 1	6 8 1	6 8 1
Carcass ID Number	0 7 5	1 2 5	1 4 5	0 6 5	1 0 5	1 2 4	0 8 5	0 7 4	0 8 4	1 2 3	0 7 3	1 4 4	1 1 4	0 5 1	0 5 2	0 5 3	0 5 4	0 5 5	0 6 1	0 6 2	0 6 3	0 6 4	0 7 1	0 7 2	0 8 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X																						
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X																						
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X																						
Intestine small, ileum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X																						
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X																						
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X	X				X		X	X		X				X	X	X				X		
Mesentery					+								+											+	
Mesothelioma malignant, metastatic, testes																									
Pancreas	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X								X		X												
Pharynx																									
Palate, squamous cell carcinoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X																						
Stomach	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X																						
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X																						
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X			X												
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X					X		X	X		X				X						X		
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X					X		X	X		X				X						X		
Pheochromocytoma benign									X				X					X		X		X			
Bilateral, pheochromocytoma benign												X		X					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 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm (continued)

Number of Days on Study	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	
Carcass ID Number	0 8 2	0 8 3	0 8 1	0 9 2	0 9 3	0 9 4	0 9 5	1 0 1	1 0 2	1 0 3	1 0 4	1 1 1	1 1 2	1 1 3	1 1 5	1 2 1	1 2 2	1 3 1	1 3 2	1 3 3	1 3 4	1 3 5	1 4 1	1 4 2	1 4 3	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X		X					X		X					X					X		X	17
Mesentery									+				+	+							+	+				8
Mesothelioma malignant, metastatic, testes																				X						1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										3
Pharynx																							+			1
Palate, squamous cell carcinoma																							X			1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										2
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										7
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										7
Pheochromocytoma benign				X			X		X							X		X		X			X			12
Bilateral, pheochromocytoma benign																									X	4

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm (continued)

Number of Days on Study	333	357	445	495	501	558	565	589	637	642	659	659	663	680	680	680	680	680	680	680	680	681	681	681	681
Carcass ID Number	075	125	145	065	105	124	085	074	084	123	073	144	114	051	052	053	054	055	061	062	063	064	071	072	081
Endocrine System (continued)																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Leukemia mononuclear			X					X		X			X												
Pars distalis, adenoma												X													
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X																						
Bilateral, c-cell, carcinoma																									
C-cell, adenoma			X												X					X					
C-cell, carcinoma																					X				
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes																									
Preputial gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																					X		X		
Carcinoma																									
Leukemia mononuclear										X															
Bilateral, adenoma							X																		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X							X															
Seminal vesicle	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear													X												
Bilateral, mesothelioma malignant																									
Bilateral, interstitial cell, adenoma			X			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										
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X													X		
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Axillary, leukemia mononuclear										X															
Iliac, leukemia mononuclear										X															
Mediastinal, leukemia mononuclear			X	X						X															
Pancreatic, leukemia mononuclear			X							X															
Renal, leukemia mononuclear										X															
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X							X	X		X				X								
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X	X						X	X		X				X								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm (continued)

Number of Days on Study	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	
Carcass ID Number	0 8 2	0 8 3	0 9 1	0 9 2	0 9 3	0 9 4	0 9 5	1 0 1	1 0 2	1 0 3	1 0 4	1 1 1	1 1 2	1 1 3	1 1 5	1 2 1	1 2 2	1 3 1	1 3 2	1 3 3	1 3 4	1 3 5	1 4 1	1 4 2	1 4 3	**Total Tissues/ Tumors**
Endocrine System (continued)																										
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma														X												1
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										4
Pars distalis, adenoma		X	X	X													X									5
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Bilateral, c-cell, carcinoma									X																	1
C-cell, adenoma			X											X												5
C-cell, carcinoma																			X							2
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, testes																				X						1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																	X	X			X					5
Carcinoma		X												X												2
Leukemia mononuclear																										1
Bilateral, adenoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										2
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Bilateral, mesothelioma malignant																				X						1
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	43
Interstitial cell, adenoma																	X									5
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										2
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Axillary, leukemia mononuclear																										1
Iliac, leukemia mononuclear																										1
Mediastinal, leukemia mononuclear						X																				4
Pancreatic, leukemia mononuclear				X		X																				4
Renal, leukemia mononuclear																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X		X																				7
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X		X																				8

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm (continued)

Number of Days on Study	3 3 4 4 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
	3 5 4 9 0 5 6 8 3 4 5 5 6 8 8 8 8 8 8 8 8 8 8 8 8 8
	3 7 5 5 1 8 5 9 7 2 9 9 3 0 0 0 0 0 0 0 0 1 1 1 1
Carcass ID Number	0 1 1 0 1 1 0 0 0 1 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0
	7 2 4 6 0 2 8 7 8 2 7 4 1 5 5 5 5 5 6 6 6 6 7 7 8
	5 5 5 5 5 4 5 4 4 3 3 4 4 1 2 3 4 5 1 2 3 4 1 2 1
Hematopoietic System (continued)	
Spleen	+ +
Leukemia mononuclear	X X X X X X X
Thymus	+ + M + + + + + M M M + + + + + + + M + + + + + +
Leukemia mononuclear	X
Integumentary System	
Mammary gland	+ + + M + + + + + + + + M + + + + + + + + + + + +
Skin	+ +
Basal cell adenoma	X
Keratoacanthoma	
Leukemia mononuclear	X
Papilloma squamous	X
Subcutaneous tissue, fibroma	
Musculoskeletal System	
Bone	
Nervous System	
Brain	+ +
Leukemia mononuclear	X X
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	X
Alveolar/bronchiolar adenoma, multiple	
Leukemia mononuclear	X X X X X X X
Nose	+ +
Trachea	+ +
Special Senses System	
Eye	+
Zymbal's gland	+ +
Carcinoma	X
Urinary System	
Kidney	+ +
Leukemia mononuclear	X X X
Urinary bladder	+ +
Leukemia mononuclear	X X X
Systemic Lesions	
Multiple organs	+ +
Leukemia mononuclear	X X X X X X X
Mesothelioma malignant	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm (continued)

Number of Days on Study	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	
Carcass ID Number	0 8 2	0 8 3	0 9 1	0 9 2	0 9 3	0 9 4	0 9 5	1 0 1	1 0 2	1 0 3	1 0 4	1 1 1	1 1 2	1 1 3	1 1 5	1 2 1	2 2 2	3 1 1	3 2 3	3 3 4	3 3 5	4 1 1	4 2 2	4 3 3	**Total Tissues/ Tumors**
Hematopoietic System (continued)																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X		X					X		X					X				X			16
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	43
Leukemia mononuclear						X																			2
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma														X											2
Keratoacanthoma								X												X					2
Leukemia mononuclear																									1
Papilloma squamous									X																2
Subcutaneous tissue, fibroma											X													X	2
Musculoskeletal System																									
Bone															+										1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																									2
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																									1
Alveolar/bronchiolar adenoma, multiple	X																								1
Leukemia mononuclear				X		X					X											X			12
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																									
Eye																									1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																									1
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X							X														5
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																									3
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X		X					X		X					X				X		X	17
Mesothelioma malignant																				X					1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm

Number of Days on Study	2 9 3	3 1 6	4 2 1	4 3 5	5 1 2	5 1 3	5 4 4	5 6 6	5 7 8	5 7 8	5 7 9	5 8 3	5 9 1	5 9 2	6 1 4	6 1 9	6 2 0	6 2 4	6 2 6	6 3 2	6 4 6	6 4 6	6 4 6	6 4 6
Carcass ID Number	3 6 5	3 3 5	3 7 5	3 5 5	3 4 5	3 1 5	3 7 4	3 4 4	3 2 5	3 6 4	3 5 4	3 1 4	3 6 3	3 5 3	3 7 3	3 3 4	3 6 2	3 3 3	3 4 2	3 1 3	3 2 4	3 5 2	3 7 1	3 7 2
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ascending colon, polyp adenomatous											X													
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic			X																					
Polyp adenomatous			X																					
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic			X																					
Leukemia mononuclear							X		X	X	X		X		X		X	X		X			X	X	X
Mesothelioma malignant, metastatic, testes						X																		
Neoplastic nodule											X										X			
Neoplastic nodule, multiple							X																	
Mesentery			+			+	+										+							
Carcinoma, metastatic			X																					
Leukemia mononuclear																	X							
Mesothelioma malignant, metastatic, testes						X																		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																	X							
Mesothelioma malignant, metastatic, testes						X																		
Pharynx		+									+			+			+						+	
Palate, papilloma squamous		X									X			X			X						X	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic			X																					
Glandular, mesothelioma malignant, metastatic, testes						X																		
Stomach, glandular	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+			+																				
Squamous cell carcinoma	X																							
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear															X			X						

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	660	668	680	800	830	840	850	881	911	881	
Carcass ID Number	361	342	222	233	311	411	511	111	112	321	**Total Tissues/ Tumors**
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	35
Intestine large	+	+	+	+	+	+	+	+	+	+	35
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	35
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	35
Ascending colon, polyp adenomatous											1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	35
Intestine small	+	+	+	+	+	+	+	+	+	+	35
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	35
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	35
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	35
Carcinoma, metastatic											1
Polyp adenomatous											1
Liver	+	+	+	+	+	+	+	+	+	+	35
Carcinoma, metastatic											1
Leukemia mononuclear	X	X	X			X	X		X	X	19
Mesothelioma malignant, metastatic, testes											1
Neoplastic nodule						X		X	X		5
Neoplastic nodule, multiple											1
Mesentery						+	+				6
Carcinoma, metastatic											1
Leukemia mononuclear											1
Mesothelioma malignant, metastatic, testes						X					2
Pancreas	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear											1
Mesothelioma malignant, metastatic, testes											1
Pharynx	+					+			+	+	9
Palate, papilloma squamous	X					X			X	X	9
Salivary glands	+	+	+	+	+	+	+	+	+	+	35
Stomach	+	+	+	+	+	+	+	+	+	+	35
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	35
Carcinoma, metastatic											1
Glandular, mesothelioma malignant, metastatic, testes											1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	34
Tongue											2
Squamous cell carcinoma											1
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear		X									3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	2 9 3	3 1 6	4 2 1	4 3 5	5 1 2	5 1 3	5 4 4	5 6 6	5 7 8	5 7 8	5 7 9	5 8 3	5 9 1	5 9 2	6 1 4	6 1 9	6 2 0	6 2 4	6 2 6	6 3 2	6 4 6	6 4 6	6 4 6	6 4 6	6 4 6
Carcass ID Number	3 6 5	3 3 5	3 7 5	3 5 5	3 4 5	3 1 5	3 7 4	3 4 4	3 2 5	3 6 4	3 5 4	3 1 4	3 6 3	3 5 3	3 7 3	3 3 4	3 6 2	3 3 3	3 3 2	3 4 3	3 1 3	3 2 4	3 5 2	3 7 1	3 7 2
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X		X						X		X	X						X	X
Mesothelioma malignant, metastatic, testes						X																			
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X		X						X		X	X					X	X	X
Pheochromocytoma benign													X									X			
Bilateral, pheochromocytoma benign								X													X				
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Pars distalis, adenoma																						X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma									X						X				X			X		X	X
C-cell, carcinoma																		X							
Follicular cell, adenoma												X							X						
General Body System																									
Tissue NOS			+																						
Carcinoma			X																						
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, multiple			X																						
Bilateral, mesothelioma malignant, metastatic, testes						X																			
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																			X	X					
Bilateral, adenoma																									
Bilateral, carcinoma									X																
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes						X																			
Seminal vesicle	M	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic			X																						
Leukemia mononuclear																	X								
Bilateral, mesothelioma malignant, metastatic, testes						X																			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	6 6 6 6 6 6 6 6 6 6 6 6 8 8 8 8 8 8 8 8 0 8 0 0 0 0 0 1 1 1	
Carcass ID Number	3 3 3 3 3 3 3 3 3 3 6 4 2 2 3 4 5 1 1 2 1 2 2 3 1 1 1 1 2 1	Total Tissues/ Tumors
Endocrine System		
Adrenal gland	+ + + + + + + + + +	35
Adrenal gland, cortex	+ + + + + + + + + +	35
Leukemia mononuclear	X X X	10
Mesothelioma malignant, metastatic, testes		1
Adrenal gland, medulla	+ + + + + + + + + +	35
Leukemia mononuclear	X X X	11
Pheochromocytoma benign	X	3
Bilateral, pheochromocytoma benign		2
Islets, pancreatic	+ + + + + + + + + +	35
Parathyroid gland	+ + + + + + + + + +	35
Pituitary gland	+ + + + + + + + + +	34
Pars distalis, adenoma		1
Thyroid gland	+ + + + + + + + + +	35
C-cell, adenoma	X X	8
C-cell, carcinoma		1
Follicular cell, adenoma	X X	4
General Body System		
Tissue NOS		1
Carcinoma		1
Genital System		
Epididymis	+ + + + + + + + + +	35
Carcinoma, metastatic, multiple		1
Bilateral, mesothelioma malignant, metastatic, testes		1
Preputial gland	+ + + + + + + + + +	35
Adenoma	X	1
Carcinoma		2
Bilateral, adenoma	X	1
Bilateral, carcinoma		1
Prostate	+ + + + + + + + + +	35
Mesothelioma malignant, metastatic, testes		1
Seminal vesicle	+ + + + + + + + + +	32
Carcinoma, metastatic		1
Leukemia mononuclear		1
Bilateral, mesothelioma malignant, metastatic, testes		1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	2 9 3	3 1 6	4 2 1	4 3 5	5 1 2	5 1 3	5 4 4	5 6 6	5 7 8	5 7 8	5 7 9	5 8 3	5 9 1	5 9 2	6 1 4	6 1 9	6 2 0	6 2 4	6 2 6	6 3 2	6 4 6	6 4 6	6 4 6	6 4 6	6 4 6
Carcass ID Number	3 6 5	3 3 5	3 7 5	3 5 5	3 4 5	3 1 5	3 7 4	3 4 4	3 2 5	3 6 4	3 5 4	3 1 4	3 6 3	3 5 3	3 7 3	3 4 4	3 6 2	3 3 3	3 3 2	3 4 3	3 1 3	3 2 4	3 5 2	3 7 1	3 7 2
Genital System (continued)																									
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic			X																						
Mesothelioma malignant														X											
Bilateral, mesothelioma malignant						X																			
Bilateral, interstitial cell, adenoma					X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Interstitial cell, adenoma			X							X															X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Axillary, leukemia mononuclear																									
Deep cervical, leukemia mononuclear																		X							X
Iliac, leukemia mononuclear																									
Mediastinal, leukemia mononuclear											X				X		X	X							
Pancreatic, leukemia mononuclear											X				X		X	X							
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear									X									X		X			X	X	X
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic			X																						
Leukemia mononuclear															X		X			X			X	X	X
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic			X																						
Leukemia mononuclear							X		X	X	X		X		X		X	X		X			X	X	X
Mesothelioma malignant, metastatic, testes						X																			
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Leukemia mononuclear															X										
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																						X	X		
Basal cell adenoma, multiple																				X				X	X
Basal cell carcinoma																					X		X		
Keratoacanthoma																			X						
Papilloma squamous									X								X								
Squamous cell carcinoma												X													
Sebaceous gland, adenoma																									
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, sarcoma														X											

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	6 6 6 6 6 6 6 6 6 6 6 6 8 8 8 8 8 8 8 8 0 8 0 0 0 0 0 1 1 1	
Carcass ID Number	3 3 3 3 3 3 3 3 3 3 6 4 2 2 3 4 5 1 1 2 1 2 2 3 1 1 1 1 2 1	**Total Tissues/ Tumors**
Genital System (continued)		
Testes	+ + + + + + + + + +	35
Carcinoma, metastatic		1
Mesothelioma malignant	X	2
Bilateral, mesothelioma malignant		1
Bilateral, interstitial cell, adenoma	X X X X X X X X X X	29
Interstitial cell, adenoma		3
Hematopoietic System		
Bone marrow	+ + + + + + + + + +	35
Leukemia mononuclear	X	1
Lymph node	+ + + + + + + + + +	35
Axillary, leukemia mononuclear	X	1
Deep cervical, leukemia mononuclear		2
Iliac, leukemia mononuclear	X	1
Mediastinal, leukemia mononuclear	X X	6
Pancreatic, leukemia mononuclear	X	5
Lymph node, mandibular	+ + + + + + + + + +	35
Leukemia mononuclear	X X X	9
Lymph node, mesenteric	+ + + + + + + + + +	35
Carcinoma, metastatic		1
Leukemia mononuclear	X X	8
Spleen	+ + + + + + + + + +	35
Carcinoma, metastatic		1
Leukemia mononuclear	X X X X X	17
Mesothelioma malignant, metastatic, testes		1
Thymus	+ + + + + + + + + +	34
Leukemia mononuclear		1
Integumentary System		
Mammary gland	+ + + + + + + + + +	34
Skin	+ + + + + + + + + +	35
Basal cell adenoma	X X X	5
Basal cell adenoma, multiple		3
Basal cell carcinoma		2
Keratoacanthoma		1
Papilloma squamous	X	3
Squamous cell carcinoma		1
Sebaceous gland, adenoma	X	1
Subcutaneous tissue, fibroma	X X	2
Subcutaneous tissue, sarcoma		1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	2 9 3	3 1 6	4 2 1	4 3 5	5 1 2	5 1 3	5 4 4	5 6 6	5 7 8	5 7 8	5 7 9	5 8 3	5 9 1	5 9 2	6 1 4	6 1 9	6 2 0	6 2 4	6 3 6	6 4 2	6 4 6	6 4 6	6 4 6	6 4 6
Carcass ID Number	3 6 5	3 3 5	3 7 5	3 5 5	3 4 5	3 1 5	3 7 4	3 4 4	3 2 5	3 6 4	3 5 4	3 1 4	3 6 3	3 5 3	3 7 3	3 3 4	3 6 2	3 3 3	3 3 2	3 4 3	3 1 3	3 2 4	3 5 2	3 7 1	3 7 2
Musculoskeletal System																								
Bone					+																			
Vertebra, osteoma					X																			
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear															X									
Cerebrum, astrocytoma malignant																								X
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								
Carcinoma, metastatic			X																					
Carcinoma, metastatic, thyroid gland																		X						
Carcinoma, metastatic, multiple, Zymbal's gland																X								
Leukemia mononuclear							X		X	X	X		X		X		X	X		X			X	X	X
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																								
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Eye																								
Harderian gland																+								
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Carcinoma												X			X	X								
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic			X																					
Leukemia mononuclear															X			X						
Mesothelioma malignant, metastatic, testes						X																		
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic			X																					
Leukemia mononuclear												X			X									
Mesothelioma malignant, metastatic, testes						X																		
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							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 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	6 6 6 6 6 6 6 6 6 6 6 6 8 8 8 8 8 8 8 8 0 8 0 0 0 0 0 1 1 1	
Carcass ID Number	3 3 3 3 3 3 3 3 3 3 6 4 2 2 3 4 5 1 1 2 1 2 2 3 1 1 1 1 2 1	**Total Tissues/ Tumors**
Musculoskeletal System		
Bone		1
Vertebra, osteoma		1
Nervous System		
Brain	+ + + + + + + + + +	35
Leukemia mononuclear		1
Cerebrum, astrocytoma malignant		1
Respiratory System		
Lung	+ + + + + + + + + +	35
Alveolar/bronchiolar adenoma	X	1
Carcinoma, metastatic		1
Carcinoma, metastatic, thyroid gland		1
Carcinoma, metastatic, multiple, Zymbal's gland		1
Leukemia mononuclear	X X X X	16
Nose	+ + + + + + + + + +	35
Squamous cell carcinoma	X	1
Trachea	+ + + + + + + + + +	35
Special Senses System		
Eye	+	1
Harderian gland		1
Zymbal's gland	+ + + + + + + + + +	35
Adenoma	X X	2
Carcinoma		3
Urinary System		
Kidney	+ + + + + + + + + +	35
Carcinoma, metastatic		1
Leukemia mononuclear		2
Mesothelioma malignant, metastatic, testes		1
Urinary bladder	+ + + + + + + + + +	35
Carcinoma, metastatic		1
Leukemia mononuclear	X	3
Mesothelioma malignant, metastatic, testes		1
Systemic Lesions		
Multiple organs	+ + + + + + + + + +	35
Leukemia mononuclear	X X X X X X X	19
Mesothelioma malignant	X	3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm

Number of Days on Study	3 2 3	3 7 2	4 0 2	4 5 1	4 6 0	4 7 2	5 0 2	5 0 5	5 0 5	5 0 6	5 3 0	5 3 4	5 5 9	5 7 0	5 7 2	5 7 4	5 7 5	5 7 7	5 7 9	5 8 3	5 8 5	5 9 5	6 0 5	6 0 6	6 1 1
Carcass ID Number	4 8 5	5 4 5	4 8 4	5 7 5	5 2 5	5 6 5	5 6 4	5 4 4	6 0 5	5 3 5	5 1 5	5 1 4	5 6 3	5 3 4	5 8 5	5 2 4	5 9 5	5 7 4	5 8 4	5 7 3	5 9 4	4 3	5 0 5	5 9 3	5 7 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Polyp adenomatous													X												
Descending colon, polyp adenomatous																									
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	A	A	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	A	A	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	A	A	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	A	A	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Leukemia mononuclear						X												X	X				X		X
Neoplastic nodule																			X						
Neoplastic nodule, multiple																									
Mesentery				+				+				+				+			+						
Mesothelioma malignant, metastatic																									
Mesothelioma malignant, metastatic, testes				X																					
Sarcoma												X													
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Pharynx				+	+			+			+					+		+	+		+			+
Squamous cell carcinoma																									
Palate, papilloma squamous					X			X			X										X				
Palate, papilloma squamous, multiple																									
Palate, squamous cell carcinoma															X		X	X							
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant												X						X							
Bilateral, schwannoma malignant																X									
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue				+			+																		
Papilloma squamous																									
Squamous cell carcinoma							X																		

Table A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	617	619	623	626	626	628	629	630	632	632	632	633	633	637	638	640	640	641	646	646	646	646	646	646	670
Carcass ID Number	562	604	603	555	602	542	513	493	554	571	583	553	592	541	504	503	512	492	502	511	533	561	614	615	523
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma						X				X											X	X			
Polyp adenomatous																									
Descending colon, polyp adenomatous																				X					
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma											X														
Leukemia mononuclear	X			X					X			X	X		X		X	X			X	X			X
Neoplastic nodule				X								X										X			X
Neoplastic nodule, multiple																									
Mesentery			+				+				+											+			
Mesothelioma malignant, metastatic																						X			
Mesothelioma malignant, metastatic, testes																									
Sarcoma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear													X												
Pharynx	+			+	+			+					+					+		+	+	+	+	+	+
Squamous cell carcinoma								X																	
Palate, papilloma squamous	X			X	X								X					X		X	X	X	X		X
Palate, papilloma squamous, multiple																								X	
Palate, squamous cell carcinoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																									
Bilateral, schwannoma malignant																									
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue												+													
Papilloma squamous																									
Squamous cell carcinoma												X													

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	6 7 0	6 7 2	6 7 3	6 7 9	6 8 0	6 8 0	6 8 0	6 8 0	6 8 0	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	
Carcass ID Number	6 1 3	5 8 2	5 8 1	5 3 1	5 5 2	5 9 1	6 0 1	6 1 1	6 1 2	4 9 1	5 0 1	5 2 1	5 2 2	5 3 2	5 5 1	**Total Tissues/ Tumors**
Alimentary System																
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	62
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	61
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	62
Adenocarcinoma																4
Polyp adenomatous																1
Descending colon, polyp adenomatous																1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	61
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	60
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	60
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	60
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	60
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Hepatocellular carcinoma																1
Leukemia mononuclear	X	X	X		X	X	X	X	X	X	X	X	X			28
Neoplastic nodule		X								X						7
Neoplastic nodule, multiple						X										1
Mesentery			+							+						11
Mesothelioma malignant, metastatic																1
Mesothelioma malignant, metastatic, testes																1
Sarcoma																1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear																1
Pharynx			+										+			23
Squamous cell carcinoma																1
Palate, papilloma squamous			X										X			16
Palate, papilloma squamous, multiple																1
Palate, squamous cell carcinoma																3
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Schwannoma malignant																2
Bilateral, schwannoma malignant																1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Papilloma squamous								X								1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Tongue				+												4
Papilloma squamous				X												1
Squamous cell carcinoma																2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	3 2 3	3 7 2	4 0 2	4 5 1	4 6 0	4 7 2	5 0 2	5 0 5	5 0 5	5 0 6	5 3 0	5 3 4	5 5 9	5 7 0	5 7 2	5 7 4	5 7 5	5 7 7	5 7 9	5 8 3	5 8 5	5 9 5	6 0 5	6 0 6	6 1 1
Carcass ID Number	4 8 5	5 4 5	4 8 4	5 7 5	5 2 5	5 6 5	5 6 4	5 4 4	6 0 5	5 3 5	5 1 5	5 1 4	5 6 3	5 3 4	5 8 5	5 2 4	5 9 5	5 7 4	5 8 4	5 7 3	5 9 4	5 4 3	5 0 5	5 9 3	5 7 2
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																		X					X		X
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									X
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																		X							X
Pheochromocytoma malignant																									
Pheochromocytoma benign													X												
Bilateral, pheochromocytoma benign					X							X										X			
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																							X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																				X					
Bilateral, C-cell, carcinoma																									
C-cell, adenoma					X										X										
C-cell, carcinoma																									
Follicular cell, adenoma																									
Follicular cell, carcinoma					X																				
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Bilateral, mesothelioma malignant, metastatic, testes				X																					
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X				X								X		
Carcinoma		X			X				X			X									X			X	
Bilateral, adenoma													X												
Bilateral, carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Schwannoma malignant																									
Seminal vesicle	M	M	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+
Schwannoma malignant, metastatic, prostate																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	6 1 7	6 1 9	6 2 3	6 2 6	6 2 6	6 2 8	6 2 9	6 3 0	6 3 2	6 3 2	6 3 2	6 3 3	6 3 3	6 3 7	6 3 8	6 4 0	6 4 0	6 4 1	6 4 6	6 4 6	6 4 6	6 4 6	6 4 6	6 4 6	6 7 0
Carcass ID Number	5 6 2	6 0 4	6 0 3	5 5 5	6 0 2	5 4 2	5 1 3	4 9 3	5 5 4	5 7 1	5 8 3	5 5 3	5 9 2	5 4 1	5 0 4	5 0 3	5 1 2	5 9 2	5 0 2	5 1 1	5 3 3	5 6 1	6 1 4	6 1 5	5 2 3
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X																					X
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X																					X
Pheochromocytoma malignant						X																			
Pheochromocytoma benign																			X	X		X		X	X
Bilateral, pheochromocytoma benign																	X								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																				X					
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
Pars distalis, adenoma																							X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
Bilateral, C-cell, carcinoma																	X								
C-cell, adenoma											X									X		X			
C-cell, carcinoma																									
Follicular cell, adenoma																							X		
Follicular cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Leukemia mononuclear																		X							
Bilateral, mesothelioma malignant, metastatic, testes																									
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Adenoma					X										X										
Carcinoma									X	X	X								X						
Bilateral, adenoma														X											
Bilateral, carcinoma																							X		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Adenoma									X																
Schwannoma malignant																				X					
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, prostate																				X					

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	6 7 0	6 7 2	6 7 3	6 7 9	6 8 0	6 8 0	6 8 0	6 8 0	6 8 0	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	
Carcass ID Number	6 1 3	5 8 2	5 8 1	5 3 1	5 5 2	5 9 1	6 0 1	6 1 1	6 1 2	4 9 1	5 0 1	5 2 1	5 2 2	5 3 2	5 5 1	**Total Tissues/ Tumors**
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear																3
Endocrine System																
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear			X													4
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear			X													5
Pheochromocytoma malignant												X				2
Pheochromocytoma benign		X			X					X	X					10
Bilateral, pheochromocytoma benign	X		X	X			X							X		9
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Adenoma																1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Pars distalis, adenoma					X											3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Bilateral, C-cell, adenoma																1
Bilateral, C-cell, carcinoma																1
C-cell, adenoma								X								6
C-cell, carcinoma										X						1
Follicular cell, adenoma																1
Follicular cell, carcinoma								X				X				3
General Body System																
None																
Genital System																
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Leukemia mononuclear																1
Bilateral, mesothelioma malignant, metastatic, testes																1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Adenoma			X					X	X		X			X		10
Carcinoma																10
Bilateral, adenoma																2
Bilateral, carcinoma																1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Adenoma																1
Schwannoma malignant																1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	57
Schwannoma malignant, metastatic, prostate																1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	3 2 3	3 7 2	4 0 2	4 5 1	4 6 0	4 7 2	5 0 2	5 0 5	5 0 5	5 0 6	5 3 0	5 3 4	5 5 9	5 7 0	5 7 2	5 7 4	5 7 5	5 7 7	5 7 9	5 8 3	5 8 5	5 9 5	6 0 5	6 0 6	6 1 1
Carcass ID Number	4 8 5	5 4 5	4 8 4	5 7 5	5 2 5	5 6 5	5 6 4	5 4 4	6 0 5	5 3 5	5 1 5	5 1 4	5 6 3	5 3 4	5 8 5	5 2 4	5 9 5	5 7 4	5 8 4	5 7 3	5 9 4	5 4 3	5 0 5	5 9 3	5 7 2
Genital System (continued)																									
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, mesothelioma malignant				X																					
Bilateral, interstitial cell, adenoma				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																									
Blood																					+				
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									X
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Iliac, leukemia mononuclear						X																			
Mediastinal, leukemia mononuclear						X												X							X
Pancreatic, leukemia mononuclear						X												X							X
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X												X							X
Squamous cell carcinoma, metastatic, tongue							X																		
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X												X							X
Spleen	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X												X	X			X			X
Thymus	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	M	+	M	M	+	M	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+
Fibroadenoma																							X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma					X												X								
Basal cell adenoma, multiple																						X	X		
Basal cell carcinoma																									
Basal cell carcinoma, multiple																									
Carcinoma																							X		
Keratoacanthoma																	X								
Papilloma squamous																									
Papilloma squamous, multiple																									
Squamous cell carcinoma					X																				
Sebaceous gland, adenoma																									
Sebaceous gland, adenoma, multiple																									
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibrous histiocytoma																									
Subcutaneous tissue, neurofibrosarcoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	6 1 7	6 1 9	6 2 3	6 2 6	6 2 6	6 2 8	6 2 9	6 3 0	6 3 2	6 3 2	6 3 2	6 3 3	6 3 3	6 3 7	6 3 8	6 4 0	6 4 0	6 4 1	6 4 6	6 4 6	6 4 6	6 4 6	6 4 6	6 4 7
Carcass ID Number	5 6 2	6 0 4	6 0 3	5 5 5	6 0 2	5 4 2	5 1 3	4 9 3	5 5 4	5 7 1	5 8 3	5 5 3	5 9 2	5 4 1	5 0 4	5 0 3	5 1 2	4 9 2	5 0 2	5 1 3	5 3 3	5 6 1	6 1 4	6 1 5	5 2 3
Genital System (continued)																									
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, mesothelioma malignant																						X			
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
Interstitial cell, adenoma									X			X													
Hematopoietic System																									
Blood																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear													X		X		X								
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Iliac, leukemia mononuclear																									
Mediastinal, leukemia mononuclear				X									X					X			X				
Pancreatic, leukemia mononuclear																		X							
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	A	M	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X								X	X				X	X			X				
Squamous cell carcinoma, metastatic, tongue																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X									X				X	X			X				
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X			X					X			X	X		X		X	X			X	X			X
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	M	M	M	+	+	+	M	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma	X	X			X	X			X									X							
Basal cell adenoma, multiple																							X	X	
Basal cell carcinoma																	X		X	X					
Basal cell carcinoma, multiple														X											
Carcinoma																									
Keratoacanthoma					X					X											X				
Papilloma squamous	X																								
Papilloma squamous, multiple												X													
Squamous cell carcinoma			X					X	X																
Sebaceous gland, adenoma												X					X		X						X
Sebaceous gland, adenoma, multiple																								X	
Subcutaneous tissue, fibroma			X					X									X								
Subcutaneous tissue, fibrous histiocytoma					X																				
Subcutaneous tissue, neurofibrosarcoma																						X			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	6 7 0	6 7 2	6 7 3	6 7 9	6 8 0	6 8 0	6 8 0	6 8 0	6 8 0	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	6 8 1	
Carcass ID Number	6 1 3	5 8 2	5 8 1	5 3 1	5 5 2	5 9 1	6 0 1	6 1 1	6 1 2	4 9 1	5 0 1	5 2 1	5 2 2	5 3 2	5 5 1	**Total Tissues/ Tumors**
Genital System (continued)																
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Bilateral, mesothelioma malignant																2
Bilateral, interstitial cell, adenoma	X	X	X	X		X	X	X	X	X	X	X		X	X	52
Interstitial cell, adenoma					X											9
Hematopoietic System																
Blood																1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Leukemia mononuclear	X		X													6
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Iliac, leukemia mononuclear																1
Mediastinal, leukemia mononuclear	X		X													9
Pancreatic, leukemia mononuclear	X															5
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	62
Leukemia mononuclear	X	X					X	X								13
Squamous cell carcinoma, metastatic, tongue																1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Leukemia mononuclear	X	X														10
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Leukemia mononuclear	X	X	X		X	X	X	X	X	X	X	X	X			28
Thymus	+	+	+	M	+	+	+	M	+	+	+	+	+	+	M	51
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	59
Fibroadenoma																1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Basal cell adenoma	X			X		X	X		X		X				X	15
Basal cell adenoma, multiple			X		X					X			X			8
Basal cell carcinoma																3
Basal cell carcinoma, multiple																1
Carcinoma																1
Keratoacanthoma	X	X			X											7
Papilloma squamous										X					X	3
Papilloma squamous, multiple			X													2
Squamous cell carcinoma		X								X	X					7
Sebaceous gland, adenoma			X	X												6
Sebaceous gland, adenoma, multiple																1
Subcutaneous tissue, fibroma	X								X							5
Subcutaneous tissue, fibrous histiocytoma																1
Subcutaneous tissue, neurofibrosarcoma																1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	3 2 3	3 7 2	4 0 2	4 5 1	4 6 0	4 7 2	5 0 2	5 0 5	5 0 5	5 0 6	5 3 0	5 3 4	5 5 9	5 7 0	5 7 2	5 7 4	5 7 5	5 7 7	5 7 9	5 8 3	5 8 5	5 9 5	6 0 5	6 0 6	6 1 1
Carcass ID Number	4 8 5	5 4 5	4 8 4	5 7 5	5 2 5	5 6 5	6 6 4	4 0 4	5 0 5	6 3 5	5 1 5	5 1 4	5 6 3	3 8 4	5 8 5	2 9 4	5 9 5	7 4 4	8 4 4	7 3 3	9 4 4	4 3 3	5 4 5	0 9 3	5 7 2
Musculoskeletal System																									
Bone			+																						
Cranium, carcinoma, metastatic, Zymbal's gland			X																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X												X							
Cerebrum, astrocytoma malignant																X									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						X
Carcinoma, metastatic, Zymbal's gland	X																								
Carcinoma, metastatic, multiple, Zymbal's gland			X																						
Leukemia mononuclear						X												X					X		X
Neoplasm NOS, metastatic, uncertain primary site																					X				
Neurofibrosarcoma, metastatic, multiple, skin																									
Squamous cell carcinoma, metastatic, multiple, skin					X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma													X												
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																						+			
Eye											+											+			
Harderian gland																									
Lacrimal gland																			+						
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X							
Carcinoma	X		X						X										X						X
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																		X							X
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, prostate																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X												X	X				X		X
Mesothelioma malignant				X																					

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	6 1 7	6 1 9	6 2 3	6 2 6	6 2 6	6 2 8	6 2 9	6 3 0	6 3 2	6 3 2	6 3 2	6 3 3	6 3 3	6 3 7	6 3 8	6 4 0	6 4 0	6 4 1	6 4 6	6 4 6	6 4 6	6 4 6	6 4 6	6 4 6	6 4 7
Carcass ID Number	5 6 2	6 0 4	6 0 3	5 5 5	6 0 2	5 4 2	5 1 3	4 9 3	5 5 4	5 7 1	5 8 3	5 5 3	5 9 2	5 4 1	5 0 4	5 0 3	5 1 2	5 9 2	5 0 2	5 1 1	5 3 3	5 6 1	6 1 4	6 1 5	5 2 3
Musculoskeletal System																									
Bone																									
Cranium, carcinoma, metastatic, Zymbal's gland																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Cerebrum, astrocytoma malignant																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma	X																								
Carcinoma, metastatic, Zymbal's gland																									
Carcinoma, metastatic, multiple, Zymbal's gland																									
Leukemia mononuclear				X					X			X	X				X	X			X				X
Neoplasm NOS, metastatic, uncertain primary site																									
Neurofibrosarcoma, metastatic, multiple, skin																						X			
Squamous cell carcinoma, metastatic, multiple, skin																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye																									
Harderian gland																									+
Lacrimal gland																									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X																		
Carcinoma																				X				X	X
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, prostate																				X					
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X			X					X			X	X		X		X	X			X	X			X
Mesothelioma malignant																						X			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	670	672	673	679	680	680	680	680	681	681	681	681	681	681	
Carcass ID Number	61 3	58 2	58 1	53 1	55 2	59 1	60 1	61 1	61 2	49 1	50 1	52 1	52 2	53 2	55 1	**Total Tissues/ Tumors**
Musculoskeletal System																
Bone																1
Cranium, carcinoma, metastatic, Zymbal's gland																1
Nervous System																
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear																2
Cerebrum, astrocytoma malignant																1
Respiratory System																
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Alveolar/bronchiolar adenoma						X										4
Carcinoma, metastatic, Zymbal's gland																1
Carcinoma, metastatic, multiple, Zymbal's gland																1
Leukemia mononuclear	X	X	X		X		X				X	X	X			20
Neoplasm NOS, metastatic, uncertain primary site																1
Neurofibrosarcoma, metastatic, multiple, skin																1
Squamous cell carcinoma, metastatic, multiple, skin																1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Squamous cell carcinoma																1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Special Senses System																
Ear											+					2
Eye																2
Harderian gland	+															2
Lacrimal gland																1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	64
Adenoma																2
Carcinoma																8
Urinary System																
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear								X								3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Schwannoma malignant, metastatic, prostate																1
Systemic Lesions																
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear	X	X	X		X	X	X	X	X	X	X	X	X			28
Mesothelioma malignant																2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm

Number of Days on Study	0 8 5	0 8 5	2 4 3	3 1 7	3 7 2	3 7 2	3 7 2	4 0 8	4 4 9	4 5 2	4 6 0	4 6 0	4 6 3	4 6 5	4 7 9	4 9 5	4 9 5	5 0 2	5 0 5	5 0 6	5 0 6	5 2 0	5 2 1	5 3 5	5 3 9
Carcass ID Number	8 1 4	8 1 5	7 9 3	8 2 5	8 1 3	8 3 5	8 8 5	8 3 4	8 9 5	8 8 3	8 4 5	8 9 4	8 3 3	8 9 3	8 5 5	8 5 4	8 6 5	8 8 4	8 5 3	8 5 2	8 9 2	9 0 5	8 4 4	8 5 1	9 0 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																			X	X					
Polyp adenomatous																									
Descending colon, polyp adenomatous																		X							
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, cystic, mucinous															X										
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma															X										
Adenocarcinoma, cystic, mucinous																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Leukemia mononuclear										X							X			X			X		X
Neoplastic nodule													X												
Neoplastic nodule, multiple																									
Mesentery																									+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx						+			+	+					+			+	+		+	+		+	
Palate, papilloma squamous						X												X	X		X	X		X	
Palate, squamous cell carcinoma															X										
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X															
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X															
Tongue																									
Papilloma squamous																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland																						X			
Leukemia mononuclear																									
Schwannoma malignant																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	5 4 3	5 4 7	5 5 1	5 5 6	5 6 1	5 6 5	5 6 5	5 6 8	5 7 0	5 7 9	5 8 3	5 9 2	6 0 0	6 1 2	6 1 3	6 2 1	6 2 4	6 4 5	6 4 6	6 4 6	6 4 6	6 5 6	6 5 9	6 8 0	6 8 0	
Carcass ID Number	9 0 1	8 4 2	8 1 1	8 2 4	8 7 5	8 7 4	9 0 3	9 0 4	8 9 1	8 6 3	8 7 3	8 6 4	8 4 3	8 6 2	8 7 2	8 3 2	8 7 1	8 3 1	8 2 1	8 2 2	8 2 3	8 8 2	8 8 1	8 4 1	8 6 1	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma										X																3
Polyp adenomatous																	X									1
Descending colon, polyp adenomatous		X																		X	X					4
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, cystic, mucinous																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																										1
Adenocarcinoma, cystic, mucinous								X																		1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma		X														X					X	X				4
Leukemia mononuclear				X	X			X					X	X	X		X	X	X	X	X	X	X	X	X	20
Neoplastic nodule										X	X	X														4
Neoplastic nodule, multiple									X									X		X						3
Mesentery	+				+									+			+								+	6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pharynx		+	+		+		+	+	+	+		+	+		+			+								20
Palate, papilloma squamous		X	X		X		X	X	X	X			X					X								15
Palate, squamous cell carcinoma															X											2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																										1
Tongue			+		+																					2
Papilloma squamous			X		X																					2
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, Zymbal's gland																										1
Leukemia mononuclear				X																						1
Schwannoma malignant														X												1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	0 8 5	0 8 5	2 4 3	3 1 7	3 7 2	3 7 2	3 7 2	4 0 8	4 4 9	4 5 2	4 6 0	4 6 0	4 6 3	4 6 5	4 7 9	4 9 5	4 9 5	5 0 2	5 0 5	5 0 6	5 0 6	5 2 0	5 2 1	5 3 5	5 3 9
Carcass ID Number	8 1 4	8 1 5	7 9 3	8 2 5	8 1 3	8 3 5	8 8 5	8 3 4	8 9 5	8 8 3	8 4 5	8 9 4	8 3 3	8 9 3	8 5 5	8 5 4	8 6 5	8 8 4	8 5 3	8 5 2	8 9 2	9 0 5	8 4 4	8 5 1	9 0 2
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																	X						X		X
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																	X						X		X
Pheochromocytoma malignant																									
Pheochromocytoma complex																	X								
Pheochromocytoma benign											X						X				X				
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																							X		
Pars distalis, adenoma																							X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																			X						
C-cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, testes													X												
Bilateral, mesothelioma malignant, metastatic, testes																									
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X					X											X				
Carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	M	M	M	+	M	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, mesothelioma malignant													X												
Bilateral, interstitial cell, adenoma									X						X	X	X	X	X	X	X		X		X
Interstitial cell, adenoma				X	X	X				X	X	X	X									X		X	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	5 4 3	5 4 7	5 5 1	5 5 6	5 6 1	5 6 5	5 6 5	5 6 8	5 7 0	5 7 9	5 8 3	5 9 2	6 0 0	6 1 2	6 1 3	6 2 1	6 2 4	6 4 5	6 4 6	6 4 6	6 4 6	6 5 6	6 5 9	6 8 0	6 8 0	
Carcass ID Number	9 0 1	8 4 2	8 1 1	8 2 4	8 7 5	8 7 4	9 0 3	9 0 4	8 9 1	8 6 3	8 7 3	8 6 4	8 4 3	8 6 2	8 7 2	8 3 2	8 7 1	8 3 1	8 2 1	8 2 2	8 2 3	8 8 2	8 8 1	8 4 1	8 6 1	**Total Tissues/ Tumors**
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X				X									X	X	X						X	9
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X				X									X	X	X						X	9
Pheochromocytoma malignant							X																			1
Pheochromocytoma complex																										1
Pheochromocytoma benign			X				X	X	X			X	X	X	X	X			X				X			14
Bilateral, pheochromocytoma benign					X																X				X	3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X													X									3
Pars distalis, adenoma																						X			X	3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma									X											X			X			4
C-cell, carcinoma																	X									1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, testes																										1
Bilateral, mesothelioma malignant, metastatic, testes						X																				1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	48
Adenoma						X			X								X				X		X			8
Carcinoma													X													1
Prostate	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, mesothelioma malignant						X																				2
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	33
Interstitial cell, adenoma								X																		10

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	0 8 5	0 8 5	2 4 3	3 1 7	3 7 2	3 7 2	3 7 2	4 0 8	4 4 9	4 5 2	4 6 0	4 6 0	4 6 3	4 6 5	4 7 9	4 9 5	4 9 5	5 0 2	5 0 5	5 0 6	5 0 6	5 2 0	5 2 1	5 3 5	5 3 9
Carcass ID Number	8 1 4	8 1 5	7 9 3	8 2 5	8 1 3	8 3 5	8 8 5	8 3 4	8 9 5	8 8 3	8 4 5	8 9 4	8 3 3	8 9 3	8 5 5	8 5 4	8 6 5	8 5 4	8 5 3	8 5 2	8 9 2	9 0 5	8 4 4	8 5 1	9 0 2
Hematopoietic System																									
Blood														+											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X															
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Axillary, carcinoma, metastatic																									
Axillary, squamous cell carcinoma, metastatic, skin																									
Mediastinal, leukemia mononuclear										X							X								X
Mediastinal, squamous cell carcinoma, metastatic																									
Pancreatic, leukemia mononuclear										X															X
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X															X
Mediastinal, carcinoma, metastatic, multiple, Zymbal's gland																						X			
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X															X
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X							X			X			X		X
Thymus	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland																						X			
Integumentary System																									
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma								X						X			X	X				X			
Basal cell adenoma, multiple																									
Basal cell carcinoma																									
Keratoacanthoma																									
Papilloma squamous											X						X								
Papilloma squamous, multiple																									
Squamous cell carcinoma																									X
Squamous cell carcinoma, multiple																									
Sebaceous gland, adenoma																									
Subcutaneous tissue, carcinoma, metastatic, Zymbal's gland																		X							
Subcutaneous tissue, fibroma				X																					
Subcutaneous tissue, sarcoma																									
Musculoskeletal System																									
Skeletal muscle			+																						
Back, schwannoma malignant, metastatic, spinal cord			X																						

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	5 4 3	5 4 7	5 5 1	5 5 6	5 6 1	5 6 5	5 6 5	5 6 8	5 7 0	5 7 9	5 8 3	5 9 2	6 0 0	6 1 2	6 1 3	6 2 1	6 2 4	6 4 5	6 4 6	6 4 6	6 4 6	6 5 6	6 5 9	6 8 0	6 8 0	
Carcass ID Number	9 0 1	8 4 2	8 1 1	8 2 4	8 7 5	8 7 4	9 0 3	9 0 4	8 9 1	8 6 3	8 7 3	8 6 4	8 4 3	8 6 2	8 7 2	8 3 2	8 7 1	8 3 1	8 2 1	8 2 2	8 2 3	8 8 2	8 8 1	8 4 1	8 6 1	**Total Tissues/ Tumors**
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear														X			X	X	X							5
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Axillary, carcinoma, metastatic										X																1
Axillary, squamous cell carcinoma, metastatic, skin												X														1
Mediastinal, leukemia mononuclear								X						X			X									6
Mediastinal, squamous cell carcinoma, metastatic										X																1
Pancreatic, leukemia mononuclear								X						X			X								X	6
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear								X					X													4
Mediastinal, carcinoma, metastatic, multiple, Zymbal's gland																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear								X					X	X					X						X	7
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X	X			X					X	X	X		X	X	X	X	X		X	X	X	19
Thymus	+	M	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Carcinoma, metastatic, Zymbal's gland																										1
Integumentary System																										
Mammary gland	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	M	M	+	+	+	+	+	M	42
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma					X	X	X										X									9
Basal cell adenoma, multiple			X	X				X	X	X	X		X	X	X	X		X	X		X	X	X	X	X	17
Basal cell carcinoma			X			X	X		X			X			X					X		X		X	X	10
Keratoacanthoma																		X			X					2
Papilloma squamous										X						X	X			X					X	7
Papilloma squamous, multiple															X											1
Squamous cell carcinoma		X				X			X					X								X			X	7
Squamous cell carcinoma, multiple	X			X				X		X		X	X	X												6
Sebaceous gland, adenoma					X													X					X			3
Subcutaneous tissue, carcinoma, metastatic, Zymbal's gland																										1
Subcutaneous tissue, fibroma									X			X														3
Subcutaneous tissue, sarcoma																					X					1
Musculoskeletal System																										
Skeletal muscle																										1
Back, schwannoma malignant, metastatic, spinal cord																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	0 8 5	0 8 5	2 4 3	3 1 7	3 7 2	3 7 2	3 7 2	4 0 8	4 4 9	4 5 2	4 6 0	4 6 0	4 6 3	4 6 5	4 7 9	4 9 5	4 9 5	5 0 2	5 0 5	5 0 6	5 0 6	5 2 0	5 2 1	5 3 5	5 3 9
Carcass ID Number	8 1 4	8 1 5	7 9 3	8 2 5	8 1 3	8 3 5	8 8 5	8 3 4	8 9 5	8 8 3	8 4 5	8 9 4	8 3 3	8 9 3	8 5 5	8 5 4	8 6 5	8 8 4	8 5 3	8 5 2	8 9 2	9 0 5	8 4 4	8 5 1	9 0 2
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Cerebrum, astrocytoma malignant															X										
Medulla, astrocytoma malignant																									
Spinal cord			+																						
Nerve, schwannoma malignant			X																						
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic, multiple, Zymbal's gland										X												X			
Leukemia mononuclear																	X						X		X
Squamous cell carcinoma, metastatic																									
Squamous cell carcinoma, metastatic, skin																									
Squamous cell carcinoma, metastatic, multiple, skin																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma						X	X	X	X		X	X				X		X	X	X	X	X		X	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X													X		X
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear										X							X			X			X		X
Mesothelioma malignant													X												

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	5 4 3	5 4 7	5 5 1	5 5 6	5 6 1	5 6 5	5 6 5	5 6 8	5 7 0	5 7 9	5 8 3	5 9 2	6 0 0	6 1 2	6 1 3	6 2 1	6 2 4	6 4 5	6 4 6	6 4 6	6 4 6	6 5 6	6 5 9	6 8 0	6 8 0	
Carcass ID Number	9 0 1	8 4 2	8 1 1	8 2 4	8 7 5	8 7 4	9 0 3	9 0 4	8 9 1	8 6 3	8 7 3	8 6 4	8 4 3	8 6 2	8 7 2	8 3 2	8 7 1	8 3 1	8 2 1	8 2 2	8 2 3	8 8 2	8 8 1	8 4 1	8 6 1	**Total Tissues/ Tumors**
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X																						1
Cerebrum, astrocytoma malignant																										1
Medulla, astrocytoma malignant																				X						1
Spinal cord																										1
Nerve, schwannoma malignant																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma											X															1
Carcinoma, metastatic, multiple, zymbal's gland																										1
Leukemia mononuclear				X				X					X	X	X		X	X	X				X	X	X	15
Squamous cell carcinoma, metastatic										X																1
Squamous cell carcinoma, metastatic, skin												X														1
Squamous cell carcinoma, metastatic, multiple, skin	X							X																		2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X	X						X					X											4
Carcinoma	X	X									X				X											17
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X				X									X		X					X		8
Renal tubule, adenoma																	X		X							2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X	X			X					X	X	X		X	X	X	X	X	X	X	X	X	20
Mesothelioma malignant						X																				2

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rates[a]	16/50 (32%)	5/35 (14%)	19/65 (29%)	17/50 (34%)
Effective rates[b]	16/47 (34%)	5/31 (16%)	19/61 (31%)	17/40 (43%)
Terminal rates[c]	13/37 (35%)	0/8 (0%)	5/11 (45%)	1/2 (50%)
First incidence (days)	637	566	460	460
Life table tests[d]	P<0.001	P=0.489	P<0.001	P<0.001
Logistic regression tests[d]	P=0.002	P=0.210N	P=0.253	P=0.012
Cochran-Armitage test[d]	P=0.125			
Fisher exact test[d]		P=0.067N	P=0.454N	P=0.278
Adrenal Medulla: Pheochromocytoma (Benign, Complex, Malignant)				
Overall rates	16/50 (32%)	5/35 (14%)	21/65 (32%)	17/50 (34%)
Effective rates	16/47 (34%)	5/31 (16%)	21/61 (34%)	17/40 (43%)
Terminal rates	13/37 (35%)	0/8 (0%)	6/11 (55%)	1/2 (50%)
First incidence (days)	637	566	460	460
Life table tests	P<0.001	P=0.489	P<0.001	P<0.001
Logistic regression tests	P=0.001	P=0.210N	P=0.134	P=0.012
Cochran-Armitage test	P=0.116			
Fisher exact test		P=0.067N	P=0.566	P=0.278
Large Intestine (Colon): Adenomatous Polyp				
Overall rates	0/50 (0%)	1/35 (3%)	2/65 (3%)	5/50 (10%)
Effective rates	0/45 (0%)	1/31 (3%)	2/59 (3%)	5/33 (15%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-[e]	579	559	502
Life table tests	P<0.001	P=0.392	P=0.193	P<0.001
Logistic regression tests	P=0.005	P=0.471	P=0.317	P=0.010
Cochran-Armitage test	P=0.003			
Fisher exact test		P=0.408	P=0.319	P=0.011
Large Intestine (Colon): Adenocarcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	4/65 (6%)	3/50 (6%)
Effective rates	0/45 (0%)	0/31 (0%)	4/58 (7%)	3/32 (9%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	-	628	505
Life table tests	P=0.002	-	P=0.025	P=0.044
Logistic regression tests	P=0.034	-	P=0.072	P=0.156
Cochran-Armitage test	P=0.020			
Fisher exact test		-	P=0.096	P=0.068
Large Intestine: Adenomatous Polyp or Adenocarcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	6/65 (9%)	8/50 (16%)
Effective rates	0/45 (0%)	1/31 (3%)	6/59 (10%)	8/33 (24%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	579	559	502
Life table tests	P<0.001	P=0.392	P=0.006	P<0.001
Logistic regression tests	P<0.001	P=0.471	P=0.030	P=0.002
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.408	P=0.030	P<0.001

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Liver: Neoplastic Nodule				
Overall rates	0/50 (0%)	6/35 (17%)	8/65 (12%)	7/50 (14%)
Effective rates	0/47 (0%)	6/31 (19%)	8/60 (13%)	7/38 (18%)
Terminal rates	0/37 (0%)	3/8 (38%)	2/11 (18%)	0/2 (0%)
First incidence (days)	-	544	579	463
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.003	P=0.002	P=0.003	P=0.003
Cochran-Armitage test	P=0.018			
Fisher exact test		P=0.003	P=0.008	P=0.003
Liver: Hepatocellular Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	1/65 (2%)	4/50 (8%)
Effective rates	0/45 (0%)	0/28 (0%)	1/53 (2%)	4/24 (17%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	-	632	547
Life table tests	P<0.001	-	P=0.446	P<0.001
Logistic regression tests	P<0.001	-	P=0.540	P=0.009
Cochran-Armitage test	P<0.001			
Fisher exact test		-	P=0.541	P=0.012
Liver: Neoplastic Nodule or Hepatocellular Carcinoma				
Overall rates	0/50 (0%)	6/35 (17%)	9/65 (14%)	11/50 (22%)
Effective rates	0/47 (0%)	6/31 (19%)	9/60 (15%)	11/38 (29%)
Terminal rates	0/37 (0%)	3/8 (38%)	2/11 (18%)	0/2 (0%)
First incidence (days)	-	544	579	463
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.002	P=0.002	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.003	P=0.004	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rates	2/50 (4%)	1/35 (3%)	4/65 (6%)	1/50 (2%)
Effective rates	2/43 (5%)	1/25 (4%)	4/47 (9%)	1/16 (6%)
Terminal rates	1/37 (3%)	1/8 (13%)	1/11 (9%)	0/2 (0%)
First incidence (days)	589	680 (T)	579	583
Life table tests	P=0.153	P=0.612	P=0.187	P=0.534
Logistic regression tests	P=0.563	P=0.698N	P=0.448	P=0.658N
Cochran-Armitage test	P=0.407			
Fisher exact test		P=0.697N	P=0.382	P=0.620
Oral Cavity (Tongue or Pharynx): Squamous Papilloma				
Overall rates	0/50 (0%)	9/35 (26%)	18/65 (28%)	15/50 (30%)
Effective rates	0/50 (0%)	9/34 (26%)	18/65 (28%)	15/47 (32%)
Terminal rates	0/37 (0%)	3/8 (38%)	1/11 (9%)	0/2 (0%)
First incidence (days)	-	316	460	372
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P<0.001	P<0.001	P<0.001

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Oral Cavity (Tongue or Pharynx): Squamous Cell Carcinoma				
Overall rates	1/50 (2%)	1/35 (3%)	6/65 (9%)	2/50 (4%)
Effective rates	1/50 (2%)	1/35 (3%)	6/65 (9%)	2/47 (4%)
Terminal rates	1/37 (3%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	680 (T)	293	502	479
Life table tests	P=0.055	P=0.557	P=0.040	P=0.117
Logistic regression tests	P=0.503	P=0.739N	P=0.141	P=0.461
Cochran-Armitage test	P=0.321			
Fisher exact test		P=0.657	P=0.110	P=0.477
Oral Cavity (Tongue or Pharynx): Squamous Papilloma or Squamous Cell Carcinoma				
Overall rates	1/50 (2%)	10/35 (29%)	24/65 (37%)	17/50 (34%)
Effective rates	1/50 (2%)	10/35 (29%)	24/65 (37%)	17/47 (36%)
Terminal rates	1/37 (3%)	3/8 (38%)	1/11 (9%)	0/2 (0%)
First incidence (days)	680 (T)	293	460	372
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P<0.001	P<0.001	P<0.001
Pharynx: Squamous Papilloma				
Overall rates	0/50 (0%)	9/35 (26%)	17/65 (26%)	15/50 (30%)
Effective rates	0/50 (0%)	9/34 (26%)	17/65 (26%)	15/47 (32%)
Terminal rates	0/37 (0%)	3/8 (38%)	1/11 (9%)	0/2 (0%)
First incidence (days)	-	316	460	372
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P<0.001	P<0.001	P<0.001
Pharynx: Squamous Cell Carcinoma				
Overall rates	1/50 (2%)	0/35 (0%)	4/65 (6%)	2/50 (4%)
Effective rates	1/47 (2%)	0/31 (0%)	4/59 (7%)	2/36 (6%)
Terminal rates	1/37 (3%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	680 (T)	-	572	479
Life table tests	P=0.033	P=0.800N	P=0.128	P=0.117
Logistic regression tests	P=0.264	P=0.800N	P=0.292	P=0.461
Cochran-Armitage test	P=0.196			
Fisher exact test		P=0.603N	P=0.260	P=0.400
Pituitary Gland (Pars Distalis): Adenoma				
Overall rates	5/49 (10%)	1/34 (3%)	3/63 (5%)	3/50 (6%)
Effective rates	5/44 (11%)	1/28 (4%)	3/53 (6%)	3/28 (11%)
Terminal rates	4/36 (11%)	0/8 (0%)	1/11 (9%)	1/2 (50%)
First incidence (days)	659	646	605	521
Life table tests	P=0.026	P=0.629N	P=0.441	P=0.015
Logistic regression tests	P=0.289	P=0.434N	P=0.512N	P=0.343
Cochran-Armitage test	P=0.560N			
Fisher exact test		P=0.240N	P=0.259N	P=0.625N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Preputial Gland: Adenoma				
Overall rates	6/49 (12%)	2/35 (6%)	12/64 (19%)	8/48 (17%)
Effective rates	6/47 (13%)	2/33 (6%)	12/63 (19%)	8/44 (18%)
Terminal rates	5/37 (14%)	1/8 (13%)	4/11 (36%)	0/2 (0%)
First incidence (days)	565	660	530	372
Life table tests	P<0.001	P=0.560	P=0.002	P<0.001
Logistic regression tests	P=0.039	P=0.466N	P=0.143	P=0.228
Cochran-Armitage test	P=0.166			
Fisher exact test		P=0.278N	P=0.270	P=0.335
Preputial Gland: Carcinoma				
Overall rates	2/49 (4%)	3/35 (9%)	11/64 (17%)	1/48 (2%)
Effective rates	2/47 (4%)	3/33 (9%)	11/63 (17%)	1/44 (2%)
Terminal rates	2/37 (5%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	680 (T)	578	372	600
Life table tests	P=0.092	P=0.107	P=0.003	P=0.379
Logistic regression tests	P=0.441N	P=0.300	P=0.056	P=0.687
Cochran-Armitage test	P=0.468N			
Fisher exact test		P=0.335	P=0.030	P=0.525N
Preputial Gland: Adenoma or Carcinoma				
Overall rates	8/49 (16%)	5/35 (14%)	23/64 (36%)	9/48 (19%)
Effective rates	8/47 (17%)	5/33 (15%)	23/63 (37%)	9/44 (20%)
Terminal rates	7/37 (19%)	1/8 (13%)	4/11 (36%)	0/2 (0%)
First incidence (days)	565	578	372	372
Life table tests	P<0.001	P=0.141	P<0.001	P<0.001
Logistic regression tests	P=0.121	P=0.547	P=0.019	P=0.232
Cochran-Armitage test	P=0.262			
Fisher exact test		P=0.539N	P=0.020	P=0.440
Salivary Glands: Malignant Schwannoma				
Overall rates	0/50 (0%)	0/35 (0%)	3/65 (5%)	0/50 (0%)
Effective rates	0/45 (0%)	0/29 (0%)	3/54 (6%)	0/27 (0%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	-	534	-
Life table tests	P=0.392	-	P=0.152	-
Logistic regression tests	P=0.654	-	P=0.214	-
Cochran-Armitage test	P=0.457			
Fisher exact test		-	P=0.158	-
Skin: Basal Cell Adenoma				
Overall rates	2/50 (4%)	8/35 (23%)	23/65 (35%)	26/50 (52%)
Effective rates	2/48 (4%)	8/33 (24%)	23/62 (37%)	26/43 (60%)
Terminal rates	1/37 (3%)	2/8 (25%)	8/11 (73%)	2/2 (100%)
First incidence (days)	659	632	460	408
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.009	P<0.001	P<0.001

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Skin: Basal Cell Carcinoma				
Overall rates	0/50 (0%)	2/35 (6%)	4/65 (6%)	10/50 (20%)
Effective rates	0/45 (0%)	2/28 (7%)	4/53 (8%)	10/23 (43%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	2/2 (100%)
First incidence (days)	-	646	637	551
Life table tests	P<0.001	P=0.063	P=0.018	P<0.001
Logistic regression tests	P<0.001	P=0.122	P=0.065	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.144	P=0.081	P<0.001
Skin: Basal Cell Adenoma or Carcinoma				
Overall rates	2/50 (4%)	9/35 (26%)	27/65 (42%)	28/50 (56%)
Effective rates	2/48 (4%)	9/33 (27%)	27/62 (44%)	28/43 (65%)
Terminal rates	1/37 (3%)	2/8 (25%)	8/11 (73%)	2/2 (100%)
First incidence (days)	659	632	460	408
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.004	P<0.001	P<0.001
Skin: Sebaceous Gland Adenoma				
Overall rates	0/50 (0%)	1/35 (3%)	7/65 (11%)	3/50 (6%)
Effective rates	0/44 (0%)	1/28 (4%)	7/52 (13%)	3/21 (14%)
Terminal rates	0/37 (0%)	1/8 (13%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	680 (T)	633	561
Life table tests	P<0.001	P=0.200	P<0.001	P=0.001
Logistic regression tests	P=0.002	P=0.200	P=0.004	P=0.026
Cochran-Armitage test	P=0.011			
Fisher exact test		P=0.389	P=0.011	P=0.030
Skin: Sebaceous Gland Adenoma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rates	2/50 (4%)	10/35 (29%)	29/65 (45%)	28/50 (56%)
Effective rates	2/48 (4%)	10/33 (30%)	29/62 (47%)	28/43 (65%)
Terminal rates	1/37 (3%)	3/8 (38%)	8/11 (73%)	2/2 (100%)
First incidence (days)	659	632	460	408
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P<0.001	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.002	P<0.001	P<0.001
Skin: Keratoacanthoma				
Overall rates	2/50 (4%)	1/35 (3%)	7/65 (11%)	2/50 (4%)
Effective rates	2/43 (5%)	1/27 (4%)	7/49 (14%)	2/16 (13%)
Terminal rates	2/37 (5%)	0/8 (0%)	1/11 (9%)	0/2 (0%)
First incidence (days)	680 (T)	626	575	645
Life table tests	P=0.004	P=0.581	P=0.006	P=0.036
Logistic regression tests	P=0.071	P=0.740	P=0.065	P=0.159
Cochran-Armitage test	P=0.106			
Fisher exact test		P=0.671N	P=0.114	P=0.295

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Skin: Squamous Papilloma				
Overall rates	2/50 (4%)	3/35 (9%)	5/65 (8%)	8/50 (16%)
Effective rates	2/47 (4%)	3/31 (10%)	5/61 (8%)	8/40 (20%)
Terminal rates	2/37 (5%)	1/8 (13%)	2/11 (18%)	1/2 (50%)
First incidence (days)	680 (T)	578	617	460
Life table tests	P<0.001	P=0.087	P=0.018	P<0.001
Logistic regression tests	P=0.001	P=0.258	P=0.107	P=0.005
Cochran-Armitage test	P=0.015			
Fisher exact test		P=0.308	P=0.340	P=0.024
Skin: Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	7/65 (11%)	13/50 (26%)
Effective rates	0/47 (0%)	1/31 (3%)	7/61 (11%)	13/40 (33%)
Terminal rates	0/37 (0%)	0/8 (0%)	2/11 (18%)	1/2 (50%)
First incidence (days)	-	591	460	539
Life table tests	P<0.001	P=0.380	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.461	P=0.018	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.397	P=0.016	P<0.001
Skin: Squamous Papilloma or Squamous Cell Carcinoma				
Overall rates	2/50 (4%)	4/35 (11%)	11/65 (17%)	19/50 (38%)
Effective rates	2/47 (4%)	4/31 (13%)	11/61 (18%)	19/40 (48%)
Terminal rates	2/37 (5%)	1/8 (13%)	3/11 (27%)	1/2 (50%)
First incidence (days)	680 (T)	578	460	460
Life table tests	P<0.001	P=0.034	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.146	P=0.011	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.166	P=0.026	P<0.001
Skin (Subcutaneous Tissue): Fibroma				
Overall rates	2/50 (4%)	2/35 (6%)	5/65 (8%)	3/50 (6%)
Effective rates	2/50 (4%)	2/33 (6%)	5/65 (8%)	3/47 (6%)
Terminal rates	2/37 (5%)	2/8 (25%)	1/11 (9%)	0/2 (0%)
First incidence (days)	680 (T)	680 (T)	623	317
Life table tests	P=0.003	P=0.143	P=0.033	P=0.054
Logistic regression tests	P=0.208	P=0.143	P=0.152	P=0.550
Cochran-Armitage test	P=0.387			
Fisher exact test		P=0.523	P=0.341	P=0.470
Skin (Subcutaneous Tissue): Fibroma or Sarcoma				
Overall rates	2/50 (4%)	3/35 (9%)	5/65 (8%)	4/50 (8%)
Effective rates	2/50 (4%)	3/33 (9%)	5/65 (8%)	4/47 (9%)
Terminal rates	2/37 (5%)	2/8 (25%)	1/11 (9%)	0/2 (0%)
First incidence (days)	680 (T)	592	623	317
Life table tests	P<0.001	P=0.050	P=0.033	P=0.008
Logistic regression tests	P=0.128	P=0.177	P=0.152	P=0.306
Cochran-Armitage test	P=0.296			
Fisher exact test		P=0.309	P=0.341	P=0.310

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Testes: Adenoma				
Overall rates	48/50 (96%)	32/35 (91%)	61/65 (94%)	43/50 (86%)
Effective rates	48/50 (96%)	32/33 (97%)	61/65 (94%)	43/47 (91%)
Terminal rates	37/37 (100%)	8/8 (100%)	10/11 (91%)	2/2 (100%)
First incidence (days)	445	421	372	317
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.352	P=0.797N	P=0.616N	P=0.595
Cochran-Armitage test	P=0.186N			
Fisher exact test		P=0.653	P=0.471N	P=0.310N
Thyroid Gland (C-cell): Adenoma				
Overall rates	5/50 (10%)	8/35 (23%)	7/65 (11%)	4/50 (8%)
Effective rates	5/48 (10%)	8/31 (26%)	7/62 (11%)	4/42 (10%)
Terminal rates	4/37 (11%)	2/8 (25%)	1/11 (9%)	0/2 (0%)
First incidence (days)	445	578	460	505
Life table tests	P=0.026	P=0.002	P=0.100	P=0.032
Logistic regression tests	P=0.493N	P=0.061	P=0.598	P=0.567
Cochran-Armitage test	P=0.307N			
Fisher exact test		P=0.069	P=0.568	P=0.585N
Thyroid Gland (C-cell): Carcinoma				
Overall rates	3/50 (6%)	1/35 (3%)	2/65 (3%)	1/50 (2%)
Effective rates	3/42 (7%)	1/18 (6%)	2/37 (5%)	1/9 (11%)
Terminal rates	3/37 (8%)	0/8 (0%)	1/11 (9%)	0/2 (0%)
First incidence (days)	680 (T)	624	640	624
Life table tests	P=0.258	P=0.663	P=0.414	P=0.375
Logistic regression tests	P=0.566	P=0.627N	P=0.642	P=0.673
Cochran-Armitage test	P=0.558			
Fisher exact test		P=0.653N	P=0.561N	P=0.552
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rates	8/50 (16%)	9/35 (26%)	9/65 (14%)	5/50 (10%)
Effective rates	8/48 (17%)	9/31 (29%)	9/62 (15%)	5/42 (12%)
Terminal rates	7/37 (19%)	2/8 (25%)	2/11 (18%)	0/2 (0%)
First incidence (days)	445	578	460	505
Life table tests	P=0.014	P=0.004	P=0.065	P=0.012
Logistic regression tests	P=0.478N	P=0.115	P=0.541N	P=0.562
Cochran-Armitage test	P=0.180N			
Fisher exact test		P=0.153	P=0.480N	P=0.369N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rates	0/50 (0%)	4/35 (11%)	1/65 (2%)	0/50 (0%)
Effective rates	0/43 (0%)	4/24 (17%)	1/46 (2%)	0/15 (0%)
Terminal rates	0/37 (0%)	2/8 (25%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	583	646	-
Life table tests	P=0.481	P=0.002	P=0.381	-
Logistic regression tests	P=0.494N	P=0.015	P=0.512	-
Cochran-Armitage test	P=0.521N			
Fisher exact test		P=0.014	P=0.517	-

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Thyroid Gland (Follicular Cell): Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	3/65 (5%)	0/50 (0%)
Effective rates	0/47 (0%)	0/31 (0%)	3/61 (5%)	0/40 (0%)
Terminal rates	0/37 (0%)	0/8 (0%)	2/11 (18%)	0/2 (0%)
First incidence (days)	-	-	460	-
Life table tests	P=0.168	-	P=0.027	-
Logistic regression tests	P=0.531	-	P=0.182	-
Cochran-Armitage test	P=0.540			
Fisher exact test		-	P=0.176	-
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rates	0/50 (0%)	4/35 (11%)	4/65 (6%)	0/50 (0%)
Effective rates	0/47 (0%)	4/31 (13%)	4/61 (7%)	0/40 (0%)
Terminal rates	0/37 (0%)	2/8 (25%)	2/11 (18%)	0/2 (0%)
First incidence (days)	-	583	460	-
Life table tests	P=0.172	P=0.002	P=0.010	-
Logistic regression tests	P=0.571N	P=0.015	P=0.100	-
Cochran-Armitage test	P=0.403N			
Fisher exact test		P=0.022	P=0.097	-
Tongue: Squamous Papilloma or Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	3/65 (5%)	2/50 (4%)
Effective rates	0/50 (0%)	1/35 (3%)	3/65 (5%)	2/47 (4%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	293	502	551
Life table tests	P=0.035	P=0.429	P=0.059	P=0.104
Logistic regression tests	P=0.326	P=0.500	P=0.185	P=0.248
Cochran-Armitage test	P=0.176			
Fisher exact test		P=0.412	P=0.177	P=0.232
Zymbal's Gland: Adenoma				
Overall rates	0/50 (0%)	2/35 (6%)	2/65 (3%)	4/50 (8%)
Effective rates	0/45 (0%)	2/28 (7%)	2/53 (4%)	4/23 (17%)
Terminal rates	0/37 (0%)	1/8 (13%)	0/11 (0%)	0/2 (0%)
First incidence (days)	-	660	577	551
Life table tests	P<0.001	P=0.023	P=0.228	P=0.004
Logistic regression tests	P=0.024	P=0.054	P=0.316	P=0.041
Cochran-Armitage test	P=0.008			
Fisher exact test		P=0.144	P=0.290	P=0.011
Zymbal's Gland: Carcinoma				
Overall rates	1/50 (2%)	3/35 (9%)	8/65 (12%)	17/50 (34%)
Effective rates	1/50 (2%)	3/33 (9%)	8/65 (12%)	17/46 (37%)
Terminal rates	0/37 (0%)	0/8 (0%)	0/11 (0%)	0/2 (0%)
First incidence (days)	501	583	323	372
Life table tests	P<0.001	P=0.128	P=0.014	P<0.001
Logistic regression tests	P<0.001	P=0.247	P=0.083	P=0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.171	P=0.040	P<0.001

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Zymbal's Gland: Adenoma or Carcinoma				
Overall rates	1/50 (2%)	5/35 (14%)	10/65 (15%)	20/50 (40%)
Effective rates	1/50 (2%)	5/33 (15%)	10/65 (15%)	20/46 (43%)
Terminal rates	0/37 (0%)	1/8 (13%)	0/11 (0%)	0/2 (0%)
First incidence (days)	501	583	323	372
Life table tests	P<0.001	P=0.007	P=0.005	P<0.001
Logistic regression tests	P<0.001	P=0.045	P=0.037	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.034	P=0.014	P<0.001
All Organs: Mononuclear Leukemia				
Overall rates	17/50 (34%)	19/35 (54%)	28/65 (43%)	20/50 (40%)
Effective rates	17/48 (35%)	19/31 (61%)	28/62 (45%)	20/42 (48%)
Terminal rates	11/37 (30%)	5/8 (63%)	9/11 (82%)	2/2 (100%)
First incidence (days)	445	544	472	452
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.004	P=0.018	P=0.053	P=0.012
Cochran-Armitage test	P=0.276			
Fisher exact test		P=0.021	P=0.202	P=0.169
All Organs: Malignant Mesothelioma				
Overall rates	1/50 (2%)	3/35 (9%)	2/65 (3%)	2/50 (4%)
Effective rates	1/47 (2%)	3/31 (10%)	2/62 (3%)	2/41 (5%)
Terminal rates	1/37 (3%)	1/8 (13%)	0/11 (0%)	0/2 (0%)
First incidence (days)	680 (T)	513	451	463
Life table tests	P=0.148	P=0.053	P=0.333	P=0.167
Logistic regression tests	P=0.575N	P=0.193	P=0.640	P=0.534
Cochran-Armitage test	P=0.509			
Fisher exact test		P=0.170	P=0.604	P=0.448
All Organs: Benign Tumors				
Overall rates	48/50 (96%)	33/35 (94%)	62/65 (95%)	46/50 (92%)
Effective rates	48/50 (96%)	33/34 (97%)	62/65 (95%)	46/47 (98%)
Terminal rates	37/37 (100%)	8/8 (100%)	11/11 (100%)	2/2 (100%)
First incidence (days)	445	316	372	317
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.093	P=0.623	P=0.760N	P=0.249
Cochran-Armitage test	P=0.440			
Fisher exact test		P=0.643	P=0.623N	P=0.523
All Organs: Malignant Tumors				
Overall	23/50 (46%)	27/35 (77%)	54/65 (83%)	45/50 (90%)
Effective	23/50 (46%)	27/35 (77%)	54/65 (83%)	45/48 (94%)
Terminal	16/37 (43%)	5/8 (63%)	9/11 (82%)	2/2 (100%)
First incidence (days)	445	293	323	243
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<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.004	P<0.001	P<0.001

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
All Organs: Benign and Malignant Tumors				
Overall	48/50 (96%)	34/35 (97%)	65/65 (100%)	48/50 (96%)
Effective	48/50 (96%)	34/35 (97%)	65/65 (100%)	48/48 (100%)
Terminal	37/37 (100%)	8/8 (100%)	11/11 (100%)	2/2 (100%)
First incidence (days)	445	293	323	243
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.018	P=0.334	P=0.138	P=0.022
Cochran-Armitage test	P=0.087			
Fisher exact test		P=0.633	P=0.187	P=0.258

(T)Terminal sacrifice
[a] Number of lesion-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 lesion-bearing animals/effective number of animals, i.e. number of animals alive at first occurance of this lesion type in any of the groups
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards lesions 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 lesions in animal group

TABLE A4a
Historical Incidence of Neoplasms of the Large Intestine in Male F344/N Rats Receiving No Treatment

Study	Incidence in Controls	
	Adenocarcinoma	Adenomatous Polyp or Adenocarcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]		
Decabromodiphenyl oxide	0/47	0/47
Chlorendic acid	0/49	0/49
Overall Historical Incidence[a]		
Total	2/1,541 (0.1%)[b]	2/1,541 (0.1%)[b]
Standard deviation	0.5%	0.5%
Range	0%–2%	0%–2%

[a] Data as of 6 March 1990, for 2-year studies
[b] Diagnosed as mucinous adenocarcinoma

TABLE A4b
Historical Incidence of Neoplasms of the Small Intestine in Male F344/N Rats Receiving No Treatment

Study	Incidence in Controls	
	Adenocarcinoma	Adenomatous Polyp or Adenocarcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]		
Decabromodiphenyl oxide	1/49[b]	1/49[b]
Chlorendic acid	0/48	0/48
Total	1/97 (1.0%)	1/97 (1.0%)
Standard deviation	1.4%	1.4%
Range	0%–2%	0%–2%
Overall Historical Incidence[c]		
Total	5/1,557 (0.3%)[d]	5/1,557 (0.3%)[d]
Standard deviation	0.8%	0.7%
Range	0%–2%	0%–2%

[a] Data as of 1 March 1989, for 2-year studies
[b] Diagnosed as carcinoma NOS.
[c] Data as of 6 March 1990, for 2-year studies
[d] Includes one carcinoma NOS and one mucinous adenocarcinoma

TABLE A4c
Historical Incidence of Liver Neoplasms in Male F344/N Rats Receiving No Treatment

Study	Incidence in Controls		
	Neoplastic Nodule	Hepatocellular Carcinoma	Neoplastic Nodule or Hepatocellular Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]			
Decabromodiphenyl oxide	1/50	1/50	2/50
Chlorendic acid	2/50	3/50	5/50
Total	3/100 (3.0%)	4/100 (4.0%)	7/100 (7.0%)
Standard deviation	1.4%	2.8%	4.2%
Range	2%–4%	2%–6%	4%–10%
Overall Historical Incidence[b]			
Total	65/1,591 (4.1%)	14/1,591 (0.9%)	78/1591 (4.9%)
Standard deviation	4.2%	1.5%	4.3%
Range	0%–12%	0%–6%	0%–14%

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies

TABLE A4d
Historical Incidence of Squamous Cell Neoplasms of the Oral Cavity[a] in Male F344/N Rats Receiving No Treatment

Study	Incidence in Controls	
	Squamous Papilloma	Squamous Cell Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[b]		
Decabromodiphenyl oxide	0/50	0/50
Chlorendic acid	0/50	0/50
Overall Historical Incidence[b]		
Total	3/1,596 (0.2%)	4/1,596 (0.3%)
Standard deviation	0.6%	0.7%
Range	0%–2%	0%–2%

[a] Includes oral mucosa, palate, soft palate, gums, and tongue
[b] Data as of 6 March 1990, for 2-year studies

TABLE A4e
Historical Incidence of Preputial Gland Neoplasms in Male F344/N Rats Receiving No Treatment

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]			
Decabromodiphenyl oxide	0/50	4/50	4/50
Chlorendic acid	0/50	1/50	1/50
Total		5/100 (5.0%)	5/100 (5.0%)
Standard deviation		4.2%	4.2%
Range		2%–8%	2%–8%
Overall Historical Incidence[b]			
Total	68/1,596 (4.3%)	49/1,596 (3.1%)	117/1596 (7.3%)
Standard deviation	5.0%	2.8%	5.2%
Range	0%–16%	0%–10%	0%–18%

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies
[c] Includes seven adenocarcinoma NOS, one squamous cell carcinoma, and 41 carcinoma NOS.

TABLE A4f
Historical Incidence of Integumentary System Basal Cell Neoplasms in Male F344/N Rats Receiving No Treatment

Study	Incidence in Controls		
	Basal Cell Tumor	Basal Cell Carcinoma	Basal Cell Tumor or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]			
Decabromodiphenyl oxide	0/50	1/50	1/50
Chlorendic acid	0/50	1/50	1/50
Total		2/100 (2.0%)	2/100 (2.0%)
Standard deviation		0.0%	0.0%
Range		2%–2%	2%–2%
Overall Historical Incidence[b]			
Total	11/1,596 (0.7%)	10/1,596 (0.6%)	21/1,596 (1.3%)
Standard deviation	1.5%	1.1%	1.9%
Range	0%–6%	0%–4%	0%–8%

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies

TABLE A4g
Historical Incidence of Integumentary System Squamous Cell Neoplasms in Male F344/N Rats Receiving No Treatment

Study	Incidence in Controls		
	Squamous Papilloma	Squamous Cell Carcinoma	Squamous Papilloma or Squamous Cell Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]			
Decabromodiphenyl oxide	1/50	1/50	2/50
Chlorendic acid	1/50	0/50	1/50
Total	2/100 (2.0%)	1/100 (1.0%)	3/100 (3.0%)
Standard deviation	0.0%	1.4%	1.4%
Range	2%–2%	0%–2%	2%–4%
Overall Historical Incidence[b]			
Total	20/1,596 (1.3%)[c]	9/1,596 (0.6%)	29/1,596 (1.8%)[c]
Standard deviation	1.5%	0.9%	1.7%
Range	0%–4%	0%–2%	0%–4%

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies
[c] Includes one papilloma NOS.

TABLE A4h
Historical Incidence of Sebaceous Gland Neoplasms in Male F344/N Rats Receiving No Treatment

Study	Incidence in Controls
Historical Incidence at Hazleton Laboratories America, Inc.[a]	
Decabromodiphenyl oxide	0/50
Chlorendic acid	0/50
Overall Historical Incidence[a]	
Total	4/1,596 (0.3%)
Standard deviation	0.7%
Range	0%–2%

[a] Data as of 6 March 1990, for 2-year studies

TABLE A4i
Historical Incidence of Zymbal's Gland Neoplasms in Male F344/N Rats Receiving No Treatment

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]			
Decabromodiphenyl oxide	0/50	0/50	0/50
Chlorendic acid	0/50	1/50	1/50
Total		1/100 (1.0%)	1/100 (1.0%)
Standard deviation		1.4%	1.4%
Range		0%–2%	0%–2%
Overall Historical Incidence[b]			
Total	1/1,596 (0.1%)[c]	18/1,596 (1.1%)	18/1,596 (1.1%)
Standard deviation	0.4%	1.8%	1.8%
Range	0%–2%	0%–8%	0%–8%

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies
[c] Diagnosed as papillary adenoma

TABLE A4j
Historical Incidence of Leukemias in Male F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls
Historical Incidence at Hazleton Laboratories America, Inc.	
Decabromodiphenyl oxide	30/50
Chlorendic acid	24/50
Total	54/100 (54.0%)
Standard deviation	8.5%
Range	48%–60%
Overall Historical Incidence	
Total	594/1,596 (37.2%)
Standard deviation	16.4%
Range	10%–72%

[a] Data as of 6 March 1990, for 2-year studies

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
9-Month interim evaluation	10	0	0	10
15-Month interim evaluation	10	10	10	10
Early deaths				
Natural deaths	5	12	24	12
Moribund kills	8	15	30	34
Accidental deaths				2
Survivors				
Terminal sacrifice	37	8	11	2
Animals examined microscopically	50	35	65	50
Alimentary System				
Intestine large, cecum	(50)	(35)	(61)	(50)
Erosion			1 (2%)	
Inflammation, acute		1 (3%)	2 (3%)	
Necrosis, focal	1 (2%)	1 (3%)		1 (2%)
Intestine large, colon	(50)	(35)	(62)	(50)
Necrosis				1 (2%)
Parasite metazoan				1 (2%)
Thrombus			1 (2%)	
Intestine large, rectum	(50)	(35)	(61)	(50)
Inflammation, acute, multifocal				1 (2%)
Intestine small, jejunum	(49)	(35)	(60)	(50)
Diverticulum				1 (2%)
Liver	(50)	(35)	(65)	(50)
Basophilic focus	27 (54%)	9 (26%)	19 (29%)	21 (42%)
Clear cell focus	1 (2%)	2 (6%)	4 (6%)	3 (6%)
Congestion		1 (3%)	3 (5%)	
Degeneration, cystic, focal	1 (2%)	3 (9%)	2 (3%)	5 (10%)
Degeneration, cystic, multifocal		2 (6%)	8 (12%)	2 (4%)
Eosinophilic focus	2 (4%)	7 (20%)	15 (23%)	23 (46%)
Granuloma	1 (2%)	1 (3%)	1 (2%)	3 (6%)
Hematopoietic cell proliferation		4 (11%)	6 (9%)	13 (26%)
Hepatodiaphragmatic nodule	7 (14%)			2 (4%)
Infarct, acute		1 (3%)	1 (2%)	
Infarct, focal				1 (2%)
Infarct, subacute				1 (2%)
Mixed cell focus	1 (2%)		1 (2%)	1 (2%)
Necrosis				1 (2%)
Necrosis, coagulative	2 (4%)		2 (3%)	
Necrosis, focal	1 (2%)		3 (5%)	2 (4%)
Necrosis, multifocal			1 (2%)	1 (2%)
Pigmentation	1 (2%)		1 (2%)	
Regeneration, diffuse			1 (2%)	3 (6%)
Regeneration, focal		2 (6%)	1 (2%)	1 (2%)
Regeneration, multifocal	1 (2%)	3 (9%)	3 (5%)	11 (22%)
Thrombus			2 (3%)	1 (2%)
Vacuolization cytoplasmic				1 (2%)
Vacuolization cytoplasmic, diffuse	1 (2%)			
Vacuolization cytoplasmic, focal	1 (2%)		3 (5%)	1 (2%)
Vacuolization cytoplasmic, multifocal	1 (2%)		3 (5%)	6 (12%)
Bile duct, cyst				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Alimentary System (continued)				
Liver (continued)				
Bile duct, hyperplasia	6 (12%)	2 (6%)	2 (3%)	
Centrilobular, degeneration, diffuse	1 (2%)	1 (3%)	7 (11%)	4 (8%)
Centrilobular, necrosis		1 (3%)		
Centrilobular, necrosis, diffuse	1 (2%)	3 (9%)	4 (6%)	2 (4%)
Mesentery	(8)	(6)	(11)	(6)
Inflammation, chronic, diffuse	1 (13%)			
Thrombus			1 (9%)	
Fat, necrosis	6 (75%)	3 (50%)	7 (64%)	6 (100%)
Pancreas	(49)	(35)	(65)	(50)
Accessory spleen	1 (2%)			
Atrophy	7 (14%)	5 (14%)	4 (6%)	3 (6%)
Acinus, hyperplasia, focal	1 (2%)			1 (2%)
Pharynx	(1)	(9)	(23)	(20)
Palate, hyperkeratosis, diffuse			1 (4%)	
Palate, hyperkeratosis, focal				3 (15%)
Palate, hyperplasia, focal			1 (4%)	
Salivary glands	(50)	(35)	(65)	(50)
Atrophy				1 (2%)
Edema			1 (2%)	
Stomach, forestomach	(49)	(35)	(65)	(50)
Acanthosis	1 (2%)		1 (2%)	1 (2%)
Erosion, focal			1 (2%)	
Erosion, multifocal			1 (2%)	
Hyperplasia, focal				1 (2%)
Inflammation, membranous, multifocal			1 (2%)	
Ulcer	1 (2%)			1 (2%)
Stomach, glandular	(49)	(34)	(65)	(50)
Erosion, focal			3 (5%)	1 (2%)
Erosion, multifocal			3 (5%)	2 (4%)
Cardiovascular System				
Heart	(50)	(35)	(65)	(50)
Cardiomyopathy, chronic	35 (70%)	25 (71%)	49 (75%)	39 (78%)
Embolus				1 (2%)
Inflammation, acute, multifocal				1 (2%)
Atrium, thrombus	2 (4%)	3 (9%)	17 (26%)	12 (24%)
Epicardium, hemorrhage, chronic			1 (2%)	
Endocrine System				
Adrenal gland, cortex	(50)	(35)	(65)	(50)
Hyperplasia, focal	1 (2%)	1 (3%)	2 (3%)	
Hypertrophy, focal	1 (2%)		1 (2%)	
Vacuolization cytoplasmic, diffuse			1 (2%)	1 (2%)
Vacuolization cytoplasmic, focal			1 (2%)	1 (2%)
Adrenal gland, medulla	(50)	(35)	(65)	(50)
Hyperplasia, focal	2 (4%)	2 (6%)	1 (2%)	2 (4%)
Hyperplasia, multifocal	2 (4%)	3 (9%)	1 (2%)	3 (6%)
Necrosis, acute			1 (2%)	
Parathyroid gland	(49)	(35)	(63)	(49)
Hyperplasia	1 (2%)			1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Endocrine System (continued)				
Pituitary gland	(49)	(34)	(63)	(50)
Pars distalis, cyst	1 (2%)		1 (2%)	1 (2%)
Pars distalis, hyperplasia, focal		1 (3%)	2 (3%)	
Thyroid gland	(50)	(35)	(65)	(50)
C-cell, hyperplasia, focal	2 (4%)		2 (3%)	3 (6%)
Follicular cell, hyperplasia, focal		1 (3%)		
General Body System				
None				
Genital System				
Preputial gland	(49)	(35)	(64)	(48)
Atrophy	10 (20%)	6 (17%)	12 (19%)	10 (21%)
Ectasia	5 (10%)	4 (11%)	15 (23%)	14 (29%)
Granuloma	1 (2%)			
Hyperplasia				1 (2%)
Hyperplasia, focal	2 (4%)			2 (4%)
Hyperplasia, squamous, focal	3 (6%)	1 (3%)	2 (3%)	2 (4%)
Inflammation, chronic				1 (2%)
Inflammation, chronic active			1 (2%)	2 (4%)
Prostate	(50)	(35)	(64)	(49)
Hemorrhage	1 (2%)			
Hyperplasia, glandular, focal	7 (14%)	4 (11%)	6 (9%)	1 (2%)
Hyperplasia, glandular, multifocal	3 (6%)	3 (9%)	2 (3%)	
Inflammation, acute	1 (2%)	1 (3%)		
Inflammation, chronic	1 (2%)		1 (2%)	
Inflammation, chronic active	2 (4%)	1 (3%)	4 (6%)	3 (6%)
Seminal vesicle	(47)	(32)	(57)	(42)
Dilatation	1 (2%)			
Hyperplasia, glandular, diffuse		1 (3%)	2 (4%)	1 (2%)
Hyperplasia, glandular, focal		1 (3%)		
Hyperplasia, glandular, multifocal		1 (3%)		
Inflammation, acute		1 (3%)		
Inflammation, chronic			1 (2%)	
Inflammation, chronic active	2 (4%)		1 (2%)	1 (2%)
Testes	(50)	(35)	(65)	(50)
Atrophy	3 (6%)	2 (6%)	8 (12%)	4 (8%)
Interstitial cell, hyperplasia	2 (4%)	1 (3%)	9 (14%)	9 (18%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Hematopoietic System				
Bone marrow	(49)	(35)	(63)	(50)
Hyperplasia	1 (2%)	14 (40%)	20 (32%)	16 (32%)
Hypoplasia			2 (3%)	
Myelofibrosis	1 (2%)		2 (3%)	1 (2%)
Lymph node	(50)	(35)	(64)	(50)
Mediastinal, angiectasis		1 (3%)		
Mediastinal, congestion			2 (3%)	
Mediastinal, hemorrhage		1 (3%)	2 (3%)	2 (4%)
Mediastinal, pigmentation		1 (3%)	2 (3%)	
Pancreatic, angiectasis		1 (3%)		
Renal, angiectasis		1 (3%)		
Lymph node, mandibular	(50)	(35)	(62)	(50)
Angiectasis		1 (3%)	1 (2%)	
Congestion				1 (2%)
Hemorrhage		1 (3%)		
Hyperplasia, lymphoid		1 (3%)	2 (3%)	2 (4%)
Hyperplasia, re cell				1 (2%)
Lymph node, mesenteric	(50)	(35)	(64)	(50)
Angiectasis	1 (2%)	1 (3%)	1 (2%)	
Atrophy		1 (3%)		
Hemorrhage	1 (2%)	2 (6%)		1 (2%)
Hyperplasia, re cell	10 (20%)	3 (9%)	7 (11%)	4 (8%)
Spleen	(50)	(35)	(64)	(50)
Atrophy	1 (2%)	3 (9%)	4 (6%)	3 (6%)
Congestion			1 (2%)	
Fibrosis, multifocal			1 (2%)	
Hematopoietic cell proliferation	1 (2%)	3 (9%)	10 (16%)	17 (34%)
Hemorrhage	1 (2%)			
Hyperplasia, reticulum cell, diffuse		1 (3%)		
Hyperplasia, reticulum cell, focal	1 (2%)	1 (3%)	1 (2%)	
Hyperplasia, reticulum cell, multifocal			1 (2%)	
Necrosis, multifocal	1 (2%)			
Pigmentation	1 (2%)			
Thymus	(43)	(34)	(51)	(43)
Atrophy		1 (3%)	1 (2%)	
Congestion		1 (3%)		
Integumentary System				
Mammary gland	(48)	(34)	(59)	(42)
Duct, ectasia			1 (2%)	
Skin	(50)	(35)	(65)	(50)
Cyst epithelial inclusion		1 (3%)	4 (6%)	1 (2%)
Hemorrhage, focal	1 (2%)			
Hair follicle, hyperplasia, basal cell, focal	1 (2%)	1 (3%)	3 (5%)	
Hair follicle, hyperplasia, basal cell, multifocal				3 (6%)
Subcutaneous tissue, edema		1 (3%)	4 (6%)	1 (2%)
Subcutaneous tissue, fibrosis, focal				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Musculoskeletal System				
Bone	(1)	(1)	(1)	
Carpal, osteopetrosis	1 (100%)			
Nervous System				
Brain	(50)	(35)	(65)	(50)
Compression		1 (3%)		
Gliosis, focal				1 (2%)
Hemorrhage	1 (2%)			
Inflammation, acute, multifocal				1 (2%)
Cerebellum, necrosis, focal		1 (3%)		
Respiratory System				
Lung	(50)	(35)	(65)	(50)
Congestion	4 (8%)	2 (6%)	3 (5%)	1 (2%)
Hemorrhage			1 (2%)	3 (6%)
Hyperplasia, lymphoid	36 (72%)	18 (51%)	39 (60%)	26 (52%)
Infiltration cellular, histiocytic	1 (2%)	1 (3%)	4 (6%)	8 (16%)
Inflammation, acute		1 (3%)	1 (2%)	
Alveolar epithelium, hyperplasia, focal			5 (8%)	4 (8%)
Alveolar epithelium, hyperplasia, multifocal			1 (2%)	
Nose	(50)	(35)	(65)	(49)
Fungus	13 (26%)	9 (26%)	13 (20%)	4 (8%)
Hyperkeratosis	2 (4%)	4 (11%)	2 (3%)	1 (2%)
Hyperplasia, glandular	1 (2%)		1 (2%)	
Inflammation, acute	15 (30%)	8 (23%)	18 (28%)	3 (6%)
Metaplasia, squamous	1 (2%)	3 (9%)	1 (2%)	
Necrosis, diffuse			1 (2%)	
Necrosis, focal			1 (2%)	
Necrosis, multifocal		1 (3%)		
Trachea	(50)	(35)	(65)	(50)
Inflammation, acute	1 (2%)			
Inflammation, chronic	1 (2%)			
Necrosis	2 (4%)			
Special Senses System				
Eye	(1)	(1)	(2)	
Atrophy			1 (50%)	
Cataract	1 (100%)			
Synechia	1 (100%)			
Retina, degeneration	1 (100%)			
Harderian gland		(1)	(2)	
Inflammation, chronic			2 (100%)	
Zymbal's gland	(50)	(35)	(64)	(50)
Abscess	1 (2%)			
Ectasia	2 (4%)	11 (31%)	8 (13%)	12 (24%)
Hyperplasia, squamous			1 (2%)	
Hyperplasia, squamous, focal		1 (3%)	5 (8%)	5 (10%)
Hypertrophy, diffuse				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Urinary System				
Kidney	(50)	(35)	(65)	(50)
Hydronephrosis	1 (2%)			
Inflammation, acute, multifocal				1 (2%)
Nephropathy, chronic	44 (88%)	28 (80%)	56 (86%)	41 (82%)
Cortex, cyst	1 (2%)			
Pelvis, inflammation, acute	1 (2%)			
Renal tubule, degeneration				2 (4%)
Renal tubule, hyperplasia, focal		1 (3%)		1 (2%)
Renal tubule, pigmentation	1 (2%)	3 (9%)		1 (2%)
Urinary bladder	(50)	(35)	(65)	(50)
Hemorrhage	2 (4%)	1 (3%)	1 (2%)	
Inflammation, acute	1 (2%)			
Transitional epithelium, hyperplasia	1 (2%)	1 (3%)	1 (2%)	1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 22-MONTH DRINKING WATER STUDY OF C.I. DIRECT BLUE 15

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
9-Month interim evaluation	10	0	0	10
15-Month interim evaluation	10	10	10	10
Early deaths				
Natural deaths	4	4	12	15
Moribund kills	6	18	31	31
Survivors				
Terminal sacrifice	40	13	22	4
Animals examined microscopically	50	35	65	50
Alimentary System				
Intestine large, colon	(50)	(35)	(65)	(50)
Polyp adenomatous			2 (3%)	
Ascending colon, polyp adenomatous			1 (2%)	
Descending colon, polyp adenomatous				1 (2%)
Intestine small, duodenum	(50)	(35)	(64)	(50)
Adenocarcinoma, cystic, mucinous			1 (2%)	
Intestine small, jejunum	(50)	(35)	(64)	(50)
Adenocarcinoma				1 (2%)
Adenocarcinoma, cystic, mucinous				2 (4%)
Liver	(50)	(35)	(65)	(50)
Hepatocellular carcinoma				1 (2%)
Histiocytic sarcoma				1 (2%)
Neoplastic nodule			2 (3%)	4 (8%)
Mesentery	(4)	(5)	(7)	(4)
Adenocarcinoma, metastatic, uterus	1 (25%)			
Pancreas	(49)	(35)	(65)	(49)
Pharynx		(4)	(21)	(12)
Palate, papilloma squamous		2 (50%)	10 (48%)	7 (58%)
Palate, papilloma squamous, multiple			1 (5%)	
Palate, squamous cell carcinoma			5 (24%)	3 (25%)
Salivary glands	(50)	(35)	(65)	(50)
Sarcoma			1 (2%)	
Stomach, forestomach	(50)	(35)	(65)	(50)
Stomach, glandular	(50)	(35)	(65)	(50)
Tongue	(2)	(3)	(6)	(9)
Papilloma squamous	2 (100%)	1 (33%)	1 (17%)	4 (44%)
Squamous cell carcinoma		1 (33%)	3 (50%)	3 (33%)
Cardiovascular System				
Heart	(50)	(35)	(65)	(50)
Schwannoma malignant			1 (2%)	
Endocrine System				
Adrenal gland, cortex	(50)	(35)	(65)	(50)
Adrenal gland, medulla	(50)	(35)	(65)	(50)
Pheochromocytoma benign	3 (6%)	2 (6%)	7 (11%)	1 (2%)
Bilateral, pheochromocytoma benign	1 (2%)	2 (6%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Endocrine System (continued)				
Islets, pancreatic	(50)	(35)	(65)	(50)
Carcinoma	1 (2%)			
Pituitary gland	(49)	(35)	(65)	(49)
Pars distalis, adenoma	18 (37%)	10 (29%)	19 (29%)	6 (12%)
Pars distalis, carcinoma			1 (2%)	
Thyroid gland	(49)	(35)	(65)	(50)
Bilateral, follicular cell, adenoma		1 (3%)		
C-cell, adenoma	10 (20%)	3 (9%)	4 (6%)	
C-cell, carcinoma	3 (6%)		3 (5%)	
Follicular cell, carcinoma				1 (2%)
General Body System				
Tissue NOS		(1)	(1)	
Genital System				
Clitoral gland	(50)	(31)	(64)	(50)
Adenoma	5 (10%)	4 (13%)	9 (14%)	8 (16%)
Carcinoma	1 (2%)	4 (13%)	10 (16%)	9 (18%)
Bilateral, adenoma		1 (3%)	3 (5%)	4 (8%)
Bilateral, carcinoma	1 (2%)	2 (6%)	2 (3%)	6 (12%)
Ovary	(50)	(35)	(64)	(50)
Adenoma		1 (3%)		
Granulosa cell tumor benign	1 (2%)			
Granulosa-theca tumor benign			1 (2%)	
Histiocytic sarcoma				1 (2%)
Uterus	(50)	(35)	(65)	(50)
Adenocarcinoma	1 (2%)			3 (6%)
Adenoma			1 (2%)	1 (2%)
Histiocytic sarcoma				1 (2%)
Leiomyosarcoma	1 (2%)			
Polyp stromal	4 (8%)	7 (20%)	9 (14%)	5 (10%)
Polyp stromal, multiple	1 (2%)	1 (3%)	3 (5%)	
Sarcoma stromal	1 (2%)		1 (2%)	2 (4%)
Cervix, sarcoma stromal, metastatic, uterus				1 (2%)
Vagina	(2)	(1)	(1)	(1)
Sarcoma stromal, metastatic, multiple, uterus				1 (100%)
Squamous cell carcinoma	1 (50%)			
Hematopoietic System				
Bone marrow	(50)	(35)	(64)	(49)
Lymph node	(50)	(35)	(65)	(50)
Iliac, carcinoma, metastatic, clitoral gland			1 (2%)	
Iliac, histiocytic sarcoma				1 (2%)
Lumbar, histiocytic sarcoma				1 (2%)
Mediastinal, histiocytic sarcoma				1 (2%)
Pancreatic, histiocytic sarcoma				1 (2%)
Renal, histiocytic sarcoma				1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Hematopoietic System (continued)				
Lymph node, mandibular	(50)	(33)	(65)	(50)
Histiocytic sarcoma				1 (2%)
Squamous cell carcinoma, metastatic, uncertain primary site		1 (3%)		
Lymph node, mesenteric	(50)	(34)	(65)	(50)
Histiocytic sarcoma				1 (2%)
Renal, mediastinal, pancreatic, mandibular, leukemia mononuclear		1 (3%)		
Spleen	(50)	(35)	(65)	(50)
Histiocytic sarcoma				1 (2%)
Sarcoma			1 (2%)	
Thymus	(47)	(31)	(63)	(45)
Integumentary System				
Mammary gland	(49)	(34)	(63)	(44)
Adenocarcinoma	4 (8%)	3 (9%)	2 (3%)	2 (5%)
Fibroadenoma	12 (24%)	2 (6%)	10 (16%)	2 (5%)
Fibroadenoma, multiple	4 (8%)	2 (6%)	6 (10%)	2 (5%)
Skin	(49)	(34)	(65)	(50)
Basal cell adenoma	1 (2%)			
Basal cell carcinoma			1 (2%)	
Keratoacanthoma	2 (4%)	1 (3%)	3 (5%)	
Papilloma squamous		2 (6%)	5 (8%)	5 (10%)
Squamous cell carcinoma			1 (2%)	
Sebaceous gland, carcinoma		1 (3%)		
Subcutaneous tissue, fibroma	3 (6%)			
Subcutaneous tissue, neurofibroma		1 (3%)		
Subcutaneous tissue, neurofibrosarcoma	1 (2%)			
Musculoskeletal System				
Bone	(13)	(4)	(12)	(7)
Cranium, carcinoma, metastatic, Zymbal's gland			1 (8%)	1 (14%)
Tibia, osteosarcoma			1 (8%)	
Skeletal muscle			(2)	(1)
Back, carcinoma, metastatic, clitoral gland			1 (50%)	
Cervical, carcinoma, metastatic, Zymbal's gland				1 (100%)
Nervous System				
Brain	(50)	(35)	(65)	(50)
Carcinoma, metastatic, Zymbal's gland				1 (2%)
Histiocytic sarcoma				1 (2%)
Cerebrum, astrocytoma malignant	1 (2%)		2 (3%)	1 (2%)
Cranial nerve, carcinoma, metastatic, Zymbal's gland				1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Respiratory System				
Lung	(50)	(35)	(65)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	2 (6%)	1 (2%)	
Carcinoma, metastatic		1 (3%)		
Carcinoma, metastatic, clitoral gland		2 (6%)		
Histiocytic sarcoma				1 (2%)
Osteosarcoma, metastatic, multiple, bone			1 (2%)	
Squamous cell carcinoma, metastatic				1 (2%)
Special Senses System				
Ear	(1)			(1)
Carcinoma, metastatic, Zymbal's gland				1 (100%)
Zymbal's gland	(49)	(35)	(64)	(50)
Adenoma		1 (3%)	5 (8%)	3 (6%)
Carcinoma		4 (11%)	7 (11%)	14 (28%)
Urinary System				
Kidney	(50)	(35)	(65)	(50)
Histiocytic sarcoma				1 (2%)
Ureter			(1)	
Transitional epithelium, papilloma			1 (100%)	
Urinary bladder	(50)	(35)	(65)	(50)
Histiocytic sarcoma				1 (2%)
Sarcoma stromal, metastatic, uterus				1 (2%)
Transitional epithelium, papilloma			1 (2%)	
Systemic Lesions				
Multiple Organs[a]	(50)	(35)	(65)	(50)
Histiocytic sarcoma				1 (2%)
Leukemia mononuclear	7 (14%)	13 (37%)	27 (42%)	15 (30%)
Mesothelioma malignant		1 (3%)		
Tumor Summary				
Total animals with primary neoplasms[b]	43	33	64	49
Total primary neoplasms	91	75	175	117
Total animals with benign neoplasms	38	27	54	33
Total benign neoplasms	68	46	105	53
Total animals with malignant neoplasms	21	24	56	48
Total malignant neoplasms	23	29	70	64
Total animals with secondary neoplasms[c]	1	4	4	5
Total secondary neoplasms	1	4	4	9
Total animals with malignant neoplasms of uncertain primary site		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 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm

Number of Days on Study	2 9 2	5 0 2	5 0 2	5 5 0	6 2 4	6 5 9	6 6 4	6 6 6	6 6 9	6 7 6	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2
Carcass ID Number	2 1 5	2 6 5	2 5 5	2 3 5	2 6 4	2 6 3	2 6 2	2 6 5	2 4 4	1 9 5	1 9 1	1 9 2	1 9 3	1 9 4	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	2 1 1	2 1 2	2 1 3	2 1 4	2 2 1	2 2 2
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X							X					X				X			
Mesentery						+														+					
Adenocarcinoma, metastatic, uterus																				X					
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue												+													
Papilloma squamous												X													
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Pheochromocytoma benign											X					X									
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																								X	
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																						X			
Pars distalis, adenoma				X	X		X							X		X		X		X	X	X		X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma						X			X			X					X						X	X	X
C-cell, carcinoma									X								X								

+: 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 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm (continued)

Number of Days on Study	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	
Carcass ID Number	2 2 3	2 2 4	2 2 5	2 3 1	2 3 2	2 3 3	2 3 4	2 4 1	2 4 2	2 4 3	2 5 1	2 5 2	2 5 3	2 5 4	2 6 1	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 8 1	2 8 2	2 8 3	2 8 4	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
Leukemia mononuclear									X						X											7
Mesentery																			+	+						4
Adenocarcinoma, metastatic, uterus																										1
Pancreas	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																						+				2
Papilloma squamous																						X				2
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear															X											1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear															X											1
Pheochromocytoma benign																					X					3
Bilateral, pheochromocytoma benign																									X	1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear																										1
Pars distalis, adenoma				X	X	X		X	X								X					X				18
Thyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma			X	X													X									10
C-cell, carcinoma																								X		3

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm (continued)

Number of Days on Study	2 9 2	5 0 0	5 0 2	5 5 0	6 2 4	6 5 9	6 6 4	6 6 6	6 6 9	6 7 6	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2
Carcass ID Number	2 1 5	2 6 5	2 5 5	2 3 5	2 6 4	2 6 3	2 6 2	2 4 5	2 4 4	1 9 5	1 9 1	1 9 2	1 9 3	1 9 4	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	2 1 1	2 1 2	2 1 3	2 1 4	2 2 1	2 2 2
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X											X						
Carcinoma																		X							
Bilateral, carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																				X					
Leiomyosarcoma																									
Polyp stromal																									X
Polyp stromal, multiple																					X				
Sarcoma stromal			X																						
Vagina																									
Squamous cell carcinoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, leukemia mononuclear																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X																X			
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X																X			
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X							X					X				X			
Thymus	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma							X																	X	X
Fibroadenoma																X		X	X	X					X
Fibroadenoma, multiple														X							X		X		
Skin	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma							X																		
Keratoacanthoma							X																		
Subcutaneous tissue, fibroma	X									X								X							
Subcutaneous tissue, neurofibrosarcoma																									
Musculoskeletal System																									
Bone		+								+									+		+		+	+	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm (continued)

Number of Days on Study	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	
Carcass ID Number	2 2 3	2 2 4	2 2 5	2 3 1	2 3 2	2 3 3	2 3 4	2 4 1	2 4 2	2 4 3	2 5 1	2 5 2	2 5 3	2 5 4	2 6 1	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 8 1	2 8 2	2 8 3	2 8 4	2 8 5	**Total Tissues/ Tumors**
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma	X									X		X														5
Carcinoma																										1
Bilateral, carcinoma						X																				1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor benign										X																1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																										1
Leiomyosarcoma							X																			1
Polyp stromal			X																X			X				4
Polyp stromal, multiple																										1
Sarcoma stromal																										1
Vagina																			+			+				2
Squamous cell carcinoma																						X				1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mediastinal, leukemia mononuclear															X											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear															X											4
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear															X											4
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear									X						X											7
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	49
Adenocarcinoma					X																					4
Fibroadenoma			X	X		X							X		X	X	X									12
Fibroadenoma, multiple												X														4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Basal cell adenoma																										1
Keratoacanthoma																X										2
Subcutaneous tissue, fibroma																										3
Subcutaneous tissue, neurofibrosarcoma																	X									1
Musculoskeletal System																										
Bone	+										+					+			+	+	+		+			13

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm (continued)

Number of Days on Study	2 9 2	5 0 0	5 0 2	5 5 0	6 2 4	6 5 9	6 6 4	6 6 6	6 6 9	6 7 6	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2
Carcass ID Number	2 1 5	2 6 5	2 5 5	2 3 5	2 6 4	2 6 3	2 6 2	2 4 5	2 4 4	1 9 5	1 9 1	1 9 2	1 9 3	1 9 4	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	2 1 1	2 1 2	2 1 3	2 1 4	2 2 1	2 2 2
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cerebrum, astrocytoma malignant		X																							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Leukemia mononuclear					X	X																X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear														+											
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X	X							X					X				X			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 0 ppm (continued)

Number of Days on Study	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	
Carcass ID Number	2 2 3	2 2 4	2 2 5	2 3 1	2 3 2	2 3 3	2 3 4	2 4 1	2 4 2	2 4 3	2 5 1	2 5 2	2 5 3	2 5 4	2 6 1	2 7 1	2 7 2	2 7 3	2 7 4	2 7 5	2 8 1	2 8 2	2 8 3	2 8 4	2 8 5	**Total Tissues/ Tumors**
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cerebrum, astrocytoma malignant																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																					X					1
Leukemia mononuclear															X											4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	49
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear									X						X											7

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm

Number of Days on Study	4 6 3	4 6 5	4 9 5	5 0 6	5 0 6	5 0 6	5 2 9	5 3 5	5 4 0	5 5 8	5 6 5	5 6 8	5 7 6	5 7 9	6 2 2	6 3 5	6 3 5	6 4 6	6 4 7	6 4 7	6 4 7	6 5 3	6 8 2	6 8 2	6 8 2
Carcass ID Number	4 4 5	4 3 5	4 6 5	4 1 4	4 1 5	4 6 4	4 4 4	4 3 4	4 5 4	4 1 3	4 6 3	4 2 5	4 0 5	4 0 4	4 6 2	4 3 2	4 3 3	4 2 4	4 4 2	4 4 3	4 6 1	4 4 1	4 0 1	4 0 2	4 0 3
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X						X	X			X	X	X	X		X			X		X				
Mesentery				+				+			+										+			+	
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear														X		X									
Pharynx	+				+																				+
Palate, papilloma squamous	X				X																				
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue								+		+															
Papilloma squamous										X															
Squamous cell carcinoma																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X												X			X					X				
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X						X	X					X			X									
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X						X	X			X		X			X									
Pheochromocytoma benign					X																				
Bilateral, pheochromocytoma benign																							X		
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X							X					X												
Pars distalis, adenoma			X															X	X	X		X			X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, follicular cell, adenoma						X																			
C-cell, adenoma																			X		X				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 3	6 8 3	6 8 3	
Carcass ID Number	4 1 1	4 1 2	4 2 1	4 2 2	4 2 3	4 3 1	4 5 1	4 5 2	4 5 3	4 5 5	**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
Liver	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear					X				X	X	13
Mesentery											5
Pancreas	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear											2
Pharynx					+						4
Palate, papilloma squamous											2
Salivary glands	+	+	+	+	+	+	+	+	+	+	35
Stomach	+	+	+	+	+	+	+	+	+	+	35
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	35
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	35
Tongue			+								3
Papilloma squamous											1
Squamous cell carcinoma			X								1
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear											4
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	35
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear									X		6
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear									X		7
Pheochromocytoma benign										X	2
Bilateral, pheochromocytoma benign				X							2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	35
Parathyroid gland	+	+	+	+	+	+	+	M	+	M	32
Pituitary gland	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear											3
Pars distalis, adenoma			X				X		X	X	10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	35
Bilateral, follicular cell, adenoma											1
C-cell, adenoma					X						3

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	4 6 3	4 6 5	4 9 5	5 0 6	5 0 6	5 0 6	5 2 9	5 3 5	5 4 0	5 5 8	5 6 5	5 6 8	5 6 6	5 7 9	5 7 2	6 2 2	6 3 5	6 3 5	6 4 6	6 4 7	6 4 7	6 4 7	6 5 3	6 8 2	6 8 2	6 8 2
Carcass ID Number	4 4 5	4 3 5	4 6 5	4 1 4	4 1 5	4 6 4	4 4 4	4 3 4	4 5 4	4 1 3	4 6 3	4 2 5	4 0 5	4 0 4	4 6 2	4 3 2	4 3 3	4 2 4	4 4 2	4 4 3	4 6 1	4 4 1	4 0 1	4 0 2	4 0 3
General Body System																										
Tissue NOS																										
Genital System																										
Clitoral gland	M	+	+	+	+	+	M	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma										X																
Carcinoma						X												X		X						
Bilateral, adenoma																			X							
Bilateral, carcinoma				X	X																					
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																X										
Leukemia mononuclear	X																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																X										
Polyp stromal									X	X	X					X								X		
Polyp stromal, multiple																										
Vagina																										
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, leukemia mononuclear							X				X		X			X					X					
Pancreatic, leukemia mononuclear							X				X										X					
Renal, leukemia mononuclear																X										
Lymph node, mandibular	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Leukemia mononuclear							X				X			X		X			X		X					
Squamous cell carcinoma, metastatic, uncertain primary site									X																	
Lymph node, mesenteric	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear											X	X	X	X		X			X		X					
Renal, mediastinal, pancreatic, mandibular, leukemia mononuclear								X																		
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X						X	X			X	X	X	X		X			X		X					
Thymus	+	+	+	+	+	+	+	M	+	M	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Leukemia mononuclear							X									X										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 3	6 8 3	6 8 3	
Carcass ID Number	4 1 1	4 1 2	4 2 1	4 2 2	4 2 3	4 3 1	4 5 1	4 5 2	4 5 3	4 5 5	**Total Tissues/ Tumors**
General Body System											
Tissue NOS							+				1
Genital System											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	31
Adenoma			X		X			X			4
Carcinoma						X					4
Bilateral, adenoma											1
Bilateral, carcinoma											2
Ovary	+	+	+	+	+	+	+	+	+	+	35
Adenoma											1
Leukemia mononuclear											1
Uterus	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear											1
Polyp stromal					X		X				7
Polyp stromal, multiple		X									1
Vagina	+										1
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	35
Lymph node	+	+	+	+	+	+	+	+	+	+	35
Mediastinal, leukemia mononuclear											5
Pancreatic, leukemia mononuclear											3
Renal, leukemia mononuclear											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	33
Leukemia mononuclear											6
Squamous cell carcinoma, metastatic, uncertain primary site											1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	34
Leukemia mononuclear											7
Renal, mediastinal, pancreatic, mandibular, leukemia mononuclear											1
Spleen	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear					X				X	X	13
Thymus	+	+	+	+	+	+	+	+	+	+	31
Leukemia mononuclear											2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	4 6 3	4 6 5	4 9 5	5 0 6	5 0 6	5 0 6	5 2 9	5 3 5	5 4 0	5 5 8	5 6 5	5 6 8	5 7 6	5 7 9	6 2 2	6 3 5	6 3 5	6 4 6	6 4 7	6 4 7	6 4 7	6 5 3	6 8 2	6 8 2	6 8 2
Carcass ID Number	4 4 5	4 3 5	4 6 5	4 1 4	4 1 5	4 6 4	4 4 4	4 3 4	4 5 4	4 1 3	4 6 3	4 2 5	4 0 5	4 0 4	4 6 2	4 3 2	4 3 3	4 2 4	4 4 2	4 4 3	4 6 1	4 4 1	4 0 1	4 0 2	4 0 3
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Adenocarcinoma					X												X								
Fibroadenoma																X		X							
Fibroadenoma, multiple														X											
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma															X										
Papilloma squamous								X																	
Sebaceous gland, carcinoma														X											
Subcutaneous tissue, neurofibroma																		X							
Musculoskeletal System																									
Bone														+											+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X									X									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma							X																		
Carcinoma, metastatic																				X					
Carcinoma, metastatic, clitoral gland					X	X																			
Leukemia mononuclear	X						X	X			X	X	X	X		X					X				
Pleura, mesothelioma malignant																				X					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																		+		+					
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X																						
Carcinoma		X	X							X								X							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X						X	X								X									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear											X														
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X						X	X			X	X	X	X		X			X		X				
Mesothelioma malignant																				X					

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 630 ppm (continued)

Number of Days on Study	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 2	6 8 3	6 8 3	6 8 3	
Carcass ID Number	4 1 1	4 1 2	4 2 1	4 2 2	4 2 3	4 3 1	4 5 1	4 5 2	4 5 3	4 5 5	**Total Tissues/ Tumors**
Integumentary System											
Mammary gland	+	+	+	+	+	+	+	+	+	+	34
Adenocarcinoma										X	3
Fibroadenoma											2
Fibroadenoma, multiple									X		2
Skin	+	+	+		+	+	+	+	+	+	34
Keratoacanthoma											1
Papilloma squamous									X		2
Sebaceous gland, carcinoma											1
Subcutaneous tissue, neurofibroma											1
Musculoskeletal System											
Bone					+			+			4
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear											2
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	35
Alveolar/bronchiolar adenoma								X			2
Carcinoma, metastatic											1
Carcinoma, metastatic, clitoral gland											2
Leukemia mononuclear									X	X	11
Pleura, mesothelioma malignant											1
Nose	+	+	+	+	+	+	+	+	+	+	35
Trachea	+	+	+	+	+	+	+	+	+	+	35
Special Senses System											
Eye					+						3
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	35
Adenoma											1
Carcinoma											4
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear											4
Urinary bladder	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear											1
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	35
Leukemia mononuclear					X				X	X	13
Mesothelioma malignant											1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	6 8 2	6 8 2	6 8 2	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	
Carcass ID Number	6 9 1	6 9 2	7 1 1	7 2 1	7 3 1	7 3 2	7 3 3	7 4 1	7 5 1	7 5 2	7 5 3	7 6 1	7 6 2	7 6 3	7 6 4	**Total Tissues/ Tumors**
Alimentary System																
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Polyp adenomatous																2
Ascending colon, polyp adenomatous									X							1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Adenocarcinoma, cystic, mucinous																1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear			X		X	X		X	X	X		X				27
Neoplastic nodule					X											2
Mesentery		+	+						+							7
Leukemia mononuclear																1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear									X			X				8
Pharynx										+						21
Palate, papilloma squamous																10
Palate, papilloma squamous, multiple																1
Palate, squamous cell carcinoma																5
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Sarcoma																1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear																1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear																1
Tongue				+												6
Papilloma squamous				X												1
Squamous cell carcinoma																3
Tooth																1
Cardiovascular System																
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear																6
Schwannoma malignant																1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	2 5 3	3 6 8	3 7 2	3 7 2	4 0 8	4 3 2	4 3 2	4 4 9	4 6 0	4 7 9	4 9 5	5 2 4	5 2 7	5 2 9	5 5 5	5 5 9	5 8 3	5 8 9	5 9 2	5 9 8	6 1 6	6 2 0	6 2 5	6 2 5	6 2 6
Carcass ID Number	6 7 5	6 6 5	6 3 5	6 8 5	6 8 4	7 1 5	7 2 5	7 4 5	6 9 5	7 0 5	6 5 5	7 0 4	7 1 3	6 6 4	6 7 4	7 4 4	7 1 4	7 2 4	6 7 3	7 6 5	6 9 4	7 5 5	7 4 2	7 4 3	6 5 4
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X					X	X					X						X
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X					X	X					X	X					X
Pheochromocytoma benign		X																		X					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear													X	X											
Pars distalis, adenoma												X		X	X	X		X				X	X		
Pars distalis, carcinoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X																	
C-cell, adenoma																									
C-cell, carcinoma																									
General Body System																									
Tissue NOS								+																	
Leukemia mononuclear								X																	
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Adenoma							X										X								
Carcinoma	X	X		X	X				X																
Bilateral, adenoma																									
Bilateral, carcinoma																								X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa-theca tumor benign																									
Leukemia mononuclear													X												
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Leukemia mononuclear																									
Polyp stromal							X									X					X			X	
Polyp stromal, multiple																									
Sarcoma stromal																									
Vagina																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	630	630	633	633	638	640	640	645	646	646	644	655	659	659	659	663	673	680	680	680	682	682	682	682	682
Carcass ID Number	64 4	65 3	64 3	70 3	73 5	71 2	72 3	70 2	67 2	70 1	66 3	66 2	69 3	69 4	73 4	73 2	72 2	64 1	68 1	64 2	65 1	65 2	66 1	67 1	68 2	68 3
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X					X					X			X		X								X	
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X					X					X			X		X						X		X	
Pheochromocytoma benign									X		X				X											X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear															X											
Pars distalis, adenoma		X									X			X					X	X						
Pars distalis, carcinoma			X																							
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																										
C-cell, adenoma													X	X		X										
C-cell, carcinoma																							X			
General Body System																										
Tissue NOS																										
Leukemia mononuclear																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma	X	X													X									X		
Carcinoma									X									X			X	X				
Bilateral, adenoma					X											X										
Bilateral, carcinoma										X																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa-theca tumor benign										X																
Leukemia mononuclear																		X								
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																						X				
Leukemia mononuclear																		X								
Polyp stromal													X									X				
Polyp stromal, multiple																				X						
Sarcoma stromal																										
Vagina																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	6 8 2	6 8 2	6 8 2	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	
Carcass ID Number	6 9 1	6 9 2	7 1 1	7 2 1	7 3 1	7 3 2	7 3 3	7 4 1	7 5 1	7 5 2	7 5 3	7 6 1	7 6 2	7 6 3	7 6 4		**Total Tissues/ Tumors**
Endocrine System																	
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Leukemia mononuclear						X			X			X					15
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Leukemia mononuclear						X			X			X					17
Pheochromocytoma benign										X							7
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		62
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Leukemia mononuclear																	3
Pars distalis, adenoma					X			X	X	X		X	X	X			19
Pars distalis, carcinoma																	1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Leukemia mononuclear																	1
C-cell, adenoma												X					4
C-cell, carcinoma		X											X				3
General Body System																	
Tissue NOS																	1
Leukemia mononuclear																	1
Genital System																	
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		64
Adenoma	X		X							X							9
Carcinoma		X															10
Bilateral, adenoma											X						3
Bilateral, carcinoma																	2
Ovary	+	+		+	+	+	+	+	+	+	+	+	+	+	+		64
Granulosa-theca tumor benign																	1
Leukemia mononuclear																	2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Adenoma																	1
Leukemia mononuclear																	1
Polyp stromal									X	X					X		9
Polyp stromal, multiple						X		X									3
Sarcoma stromal				X													1
Vagina															+		1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	2 5 2 3	3 6 3 8	3 7 2 2	3 7 0 2	4 0 8 8	4 3 2 2	4 3 2 2	4 4 2 9	4 6 0 0	4 7 0 9	4 9 5 5	5 2 4 4	5 2 7 3	5 2 9 1	5 5 5 3	5 5 9 3	5 8 3 9	5 8 9 2	5 9 9 8	6 1 6 6	6 2 0 5	6 2 2 5	6 2 5 5	6 2 6 4
Carcass ID Number	6 7 5 5	6 6 5 5	6 3 5 5	6 8 5 5	6 8 4 4	7 1 4 5	7 2 5 5	7 4 5 5	6 9 5 5	7 0 5 5	6 5 5 5	7 0 4 4	7 1 3 3	6 6 4 4	6 7 4 4	7 4 1 4	7 1 4 4	7 2 4 4	6 7 3 3	7 6 5 5	6 9 4 4	7 5 5 5	7 4 2 2	4 4 3 3	6 5 4 4
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																			X					X
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deep cervical, leukemia mononuclear																								
Iliac, carcinoma, metastatic, clitoral gland		X																						
Iliac, leukemia mononuclear																					X			
Lumbar, leukemia mononuclear																					X			
Mediastinal, leukemia mononuclear								X					X	X					X		X			X
Pancreatic, leukemia mononuclear								X					X						X		X			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X					X	X					X		X			X
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X					X	X					X	X	X			X
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X					X	X	X			X	X	X	X			X
Sarcoma																		X						
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								
Integumentary System																								
Mammary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																						X		
Fibroadenoma															X			X						
Fibroadenoma, multiple																	X							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																								
Keratoacanthoma																				X				
Papilloma squamous																								
Squamous cell carcinoma																								
Musculoskeletal System																								
Bone						+			+															
Cranium, carcinoma, metastatic, Zymbal's gland						X																		
Tibia, osteosarcoma																								
Skeletal muscle																								
Back, carcinoma, metastatic, clitoral gland																								
Diaphragm, leukemia mononuclear																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	630	630	633	633	633	640	640	645	646	646	654	659	659	659	666	673	680	680	682	682	682	682	682	682	682
Carcass ID Number	6644	6543	6403	7033	7035	7112	7233	7022	7072	7001	6663	6622	6693	7054	7344	7222	6441	6811	6424	6515	6521	6661	6711	6822	6883
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deep cervical, leukemia mononuclear																			X						
Iliac, carcinoma, metastatic, clitoral gland																									
Iliac, leukemia mononuclear		X																							
Lumbar, leukemia mononuclear		X																							
Mediastinal, leukemia mononuclear	X	X															X								
Pancreatic, leukemia mononuclear	X	X										X													
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X					X					X			X				X						
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X					X					X			X		X		X			X			
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X					X					X		X	X		X		X			X		X	X
Sarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X										X								
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Fibroadenoma				X				X	X						X					X			X	
Fibroadenoma, multiple		X					X			X															
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																								X	
Keratoacanthoma	X																X								
Papilloma squamous																X							X		X
Squamous cell carcinoma															X										
Musculoskeletal System																									
Bone																			+			+		+	
Cranium, carcinoma, metastatic, Zymbal's gland																									
Tibia, osteosarcoma																									
Skeletal muscle																	+	+							
Back, carcinoma, metastatic, clitoral																		X							
Diaphragm, leukemia mononuclear																	X								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	6 8 2	6 8 2	6 8 2	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	
Carcass ID Number	6 9 1	6 9 2	7 1 1	7 2 1	7 3 1	7 3 2	7 3 3	7 4 1	7 5 1	7 5 2	7 5 3	7 6 1	7 6 2	7 6 3	7 6 4	**Total Tissues/ Tumors**
Hematopoietic System																
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	64
Leukemia mononuclear																2
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Deep cervical, leukemia mononuclear																1
Iliac, carcinoma, metastatic, clitoral gland																1
Iliac, leukemia mononuclear																2
Lumbar, leukemia mononuclear																2
Mediastinal, leukemia mononuclear												X				10
Pancreatic, leukemia mononuclear			X													8
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear			X			X			X			X				15
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear			X			X			X			X				19
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Leukemia mononuclear			X		X	X		X	X	X		X				27
Sarcoma																1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Leukemia mononuclear						X										3
Integumentary System																
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	63
Adenocarcinoma			X													2
Fibroadenoma				X				X								10
Fibroadenoma, multiple			X				X									6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	65
Basal cell carcinoma																1
Keratoacanthoma																3
Papilloma squamous		X											X			5
Squamous cell carcinoma																1
Musculoskeletal System																
Bone		+					+	+			+		+	+	+	12
Cranium, carcinoma, metastatic, Zymbal's gland																1
Tibia, osteosarcoma														X		1
Skeletal muscle																2
Back, carcinoma, metastatic, clitoral gland																1
Diaphragm, leukemia mononuclear																1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	2 5 3	3 6 8	3 7 2	3 7 2	4 0 8	4 3 2	4 3 2	4 4 9	4 6 0	4 7 9	4 9 5	5 2 4	5 2 7	5 2 9	5 5 5	5 5 9	5 8 3	5 8 9	5 9 2	5 9 8	6 1 6	6 2 0	6 2 5	6 2 5	6 2 6
Carcass ID Number	6 7 5	6 6 5	6 3 5	6 8 5	6 8 4	7 1 5	7 2 5	7 4 5	6 9 5	7 0 5	6 5 5	7 0 4	7 1 3	7 6 4	6 7 4	7 4 4	7 1 4	7 2 4	7 7 3	6 7 5	6 9 4	7 5 5	7 4 2	7 4 3	6 5 4
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear													X												
Cerebrum, astrocytoma malignant																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Leukemia mononuclear								X					X	X	X					X	X	X			X
Osteosarcoma, metastatic, multiple, bone																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+																								
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma						X														X					
Carcinoma						X					X						X		X						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																					X				X
Ureter																									
Transitional epithelium, papilloma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Transitional epithelium, papilloma															X										
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 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	630	630	633	633	638	640	640	645	646	646	645	659	659	659	666	673	680	680	682	682	682	682	682	682	682
Carcass ID Number	644	653	643	703	735	712	723	702	707	701	663	662	693	754	733	722	741	811	842	851	852	861	871	882	883
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X													X										
Cerebrum, astrocytoma malignant					X															X					
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Leukemia mononuclear	X	X					X					X			X		X		X					X	
Osteosarcoma, metastatic, multiple, bone																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye						+					+												+		
Zymbal's gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X			X		X					
Carcinoma							X				X														
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X													X		X								
Ureter																									
Transitional epithelium, papilloma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear															X										
Transitional epithelium, papilloma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X					X					X		X	X		X		X			X		X	X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 1,250 ppm (continued)

Number of Days on Study	6 8 2	6 8 2	6 8 2	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	6 8 3	
Carcass ID Number	6 9 1	6 9 2	7 1 1	7 2 1	7 3 1	7 3 2	7 3 3	7 4 1	7 5 1	7 5 2	7 5 3	7 6 1	7 6 2	7 6 3	7 6 4		**Total Tissues/ Tumors**
Nervous System																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Leukemia mononuclear																	4
Cerebrum, astrocytoma malignant																	2
Respiratory System																	
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Alveolar/bronchiolar adenoma																	1
Leukemia mononuclear						X			X			X					19
Osteosarcoma, metastatic, multiple, bone														X			1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		64
Special Senses System																	
Eye																	4
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		64
Adenoma																	5
Carcinoma			X														7
Urinary System																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Leukemia mononuclear						X						X					7
Ureter								+									1
Transitional epithelium, papilloma								X									1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Leukemia mononuclear																	1
Transitional epithelium, papilloma																	1
Systemic Lesions																	
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		65
Leukemia mononuclear			X		X	X		X	X	X		X					27

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	5 8 1	5 8 5	5 8 5	5 8 5	5 9 2	5 9 2	6 0 0	6 0 0	6 0 7	6 1 3	6 1 9	6 2 0	6 2 3	6 2 4	6 2 4	6 3 5	6 4 5	6 4 6	6 4 6	6 4 6	6 4 6	6 8 2	6 8 2	6 8 2	6 8 2	
Carcass ID Number	1 0 1 3	0 9 8 4	0 9 8 5	0 9 9 2	1 0 2 2	1 0 4 2	0 9 6 4	1 0 2 1	0 9 7 2	1 0 3 3	0 9 6 3	1 0 3 2	0 9 8 3	0 9 7 1	1 0 4 1	1 0 0 2	0 9 6 2	0 9 8 2	0 9 9 1	1 0 0 1	1 0 3 1	0 9 6 1	0 9 8 1	1 0 1 1	1 0 1 2	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Descending colon, polyp adenomatous																				X						1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																				X						1
Adenocarcinoma, cystic, mucinous			X																							2
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																										1
Histiocytic sarcoma																										1
Leukemia mononuclear		X			X		X					X	X	X	X	X			X	X	X		X		X	15
Neoplastic nodule			X	X											X										X	4
Mesentery					+													+								4
Pancreas	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear													X													1
Pharynx		+			+			+	+					+												12
Palate, papilloma squamous		X			X			X	X					X												7
Palate, squamous cell carcinoma																										3
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue		+	+												+							+	+	+		9
Papilloma squamous		X																						X		4
Squamous cell carcinoma			X												X								X			3
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear							X						X													3

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	2 9 6	3 1 6	3 3 9	3 5 9	3 7 2	4 0 2	4 0 8	4 0 8	4 5 3	4 6 3	4 6 6	4 7 4	4 8 4	4 8 6	4 9 5	4 9 5	5 0 2	5 0 2	5 1 3	5 1 3	5 2 7	5 3 4	5 4 7	5 6 4	5 7 8
Carcass ID Number	10 02 55	09 04 55	10 02 44	10 03 55	09 04 44	10 04 55	09 03 55	09 09 55	09 93 44	10 03 44	09 06 55	10 03 33	10 04 55	10 04 44	09 05 11	09 03 44	09 07 55	09 01 55	09 09 44	10 91 44	09 01 44	09 07 44	10 02 33	09 04 33	09 09 33	09 09 73
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear									X										X						
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear									X										X						
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma						X																			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, carcinoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X			X	X												
Carcinoma					X	X		X		X	X									X		X			
Bilateral, adenoma																									
Bilateral, carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																					X				
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Adenoma																									
Histiocytic sarcoma																					X				
Polyp stromal									X	X				X		X									
Sarcoma stromal							X																		
Cervix, sarcoma stromal, metastatic, uterus																									
Vagina																									
Sarcoma stromal, metastatic, multiple, uterus																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	5 8 1	5 8 5	5 8 5	5 8 5	5 9 2	5 9 2	6 0 0	6 0 0	6 0 7	6 1 3	6 1 9	6 2 0	6 2 3	6 2 4	6 2 4	6 3 5	6 4 5	6 4 6	6 4 6	6 4 6	6 4 6	6 8 2	6 8 2	6 8 2	6 8 2	
Carcass ID Number	1 0 1 3	0 9 8 4	0 9 8 5	0 9 9 2	1 0 2 2	1 0 0 2	0 9 4 4	0 9 6 2	1 0 2 1	0 9 7 2	1 0 0 3	0 9 6 3	1 0 3 2	0 9 8 3	0 9 7 1	1 0 4 1	1 0 0 2	0 9 6 2	0 9 8 2	0 9 9 1	1 0 0 1	1 0 3 1	0 9 6 1	0 9 8 1	1 0 1 1	1 0 1 2	**Total Tissues/ Tumors**
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X		X								X	X				X	X					8
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear					X		X								X	X				X	X					8
Pheochromocytoma benign													X													1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma					X									X			X			X		X				6
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, carcinoma																		X								1
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma									X											X	X		X		X	8
Carcinoma												X	X													9
Bilateral, adenoma						X				X				X		X										4
Bilateral, carcinoma		X	X	X				X									X		X							6
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma									X	X				X												3
Adenoma																							X			1
Histiocytic sarcoma																										1
Polyp stromal																								X		5
Sarcoma stromal						X																				2
Cervix, sarcoma stromal, metastatic, uterus						X																				1
Vagina						+																				1
Sarcoma stromal, metastatic, multiple, uterus						X																				1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	2 9 6	3 1 6	3 3 9	3 5 9	3 7 2	4 0 2	4 0 8	4 0 8	4 5 3	4 6 3	4 6 6	4 7 4	4 8 4	4 8 6	4 9 5	4 9 5	5 0 2	5 0 2	5 1 3	5 1 3	5 2 7	5 3 4	5 4 7	5 6 4	5 7 8
Carcass ID Number	1 0 2 5	0 9 4 5	1 0 2 4	1 0 3 5	0 9 4 4	1 0 4 5	0 9 3 5	0 9 9 5	0 9 3 4	1 0 3 4	0 9 6 5	1 0 0 3	1 0 4 5	1 0 4 4	0 9 5 1	1 0 3 4	0 9 7 5	1 0 1 5	0 9 9 4	1 0 1 4	0 9 7 4	1 0 2 3	1 0 4 3	0 9 9 3	0 9 7 3
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Leukemia mononuclear																									
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Iliac, histiocytic sarcoma																					X				
Lumbar, histiocytic sarcoma																					X				
Mediastinal, histiocytic sarcoma																					X				
Mediastinal, leukemia mononuclear									X									X							
Pancreatic, histiocytic sarcoma																					X				
Pancreatic, leukemia mononuclear																									
Renal, histiocytic sarcoma																					X				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																					X				
Leukemia mononuclear									X									X							
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																					X				
Leukemia mononuclear									X									X							
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																					X				
Leukemia mononuclear									X									X							
Thymus	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	M	M	+	+	+
Leukemia mononuclear																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	M
Adenocarcinoma																							X		
Fibroadenoma																		X							
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																									
Musculoskeletal System																									
Bone									+				+									+			
Cranium, carcinoma, metastatic, Zymbal's gland													X												
Skeletal muscle													+												
Cervical, carcinoma, metastatic, Zymbal's gland													X												

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	5 8 1	5 8 5	5 8 5	5 8 5	5 9 2	5 9 2	6 0 0	6 0 0	6 0 7	6 1 3	6 1 9	6 2 0	6 2 3	6 2 4	6 2 4	6 3 5	6 4 5	6 4 6	6 4 6	6 4 6	6 4 6	6 8 2	6 8 2	6 8 2	6 8 2	
Carcass ID Number	1 0 1 3	0 9 8 4	0 9 8 5	0 9 9 2	1 0 2 2	1 0 4 2	0 9 6 4	1 0 2 1	1 0 9 2	0 9 7 3	1 0 3 3	0 9 6 3	1 0 3 2	0 9 8 3	0 9 7 1	1 0 4 1	1 0 0 2	0 9 6 2	0 9 8 2	0 9 9 1	1 0 0 1	1 0 3 1	0 9 6 1	0 9 8 1	1 0 1 1	1 0 1 2	**Total Tissues/ Tumors**
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear														X												1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Iliac, histiocytic sarcoma																										1
Lumbar, histiocytic sarcoma																										1
Mediastinal, histiocytic sarcoma																										1
Mediastinal, leukemia mononuclear														X			X									4
Pancreatic, histiocytic sarcoma																										1
Pancreatic, leukemia mononuclear														X												1
Renal, histiocytic sarcoma																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear					X		X							X			X				X					7
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear					X		X							X			X				X					7
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear		X			X		X					X	X	X	X	X			X	X	X		X		X	15
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear														X												1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	M	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	44
Adenocarcinoma																						X				2
Fibroadenoma																	X									2
Fibroadenoma, multiple						X												X								2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papilloma squamous									X	X			X						X		X					5
Musculoskeletal System																										
Bone																	+			+				+	+	7
Cranium, carcinoma, metastatic, Zymbal's gland																										1
Skeletal muscle																										1
Cervical, carcinoma, metastatic, Zymbal's gland																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	2 9 6	3 1 6	3 3 9	3 5 9	3 7 2	4 0 2	4 0 8	4 0 8	4 5 3	4 6 3	4 6 6	4 7 4	4 8 4	4 8 6	4 9 5	4 9 5	5 0 2	5 0 2	5 1 3	5 1 3	5 2 7	5 3 4	5 4 7	5 6 4	5 7 8
Carcass ID Number	1 0 2 5	0 9 4 5	1 0 2 4	1 0 3 5	0 9 4 4	1 0 4 5	0 9 3 5	0 9 9 5	0 9 3 4	1 0 3 4	0 9 6 5	1 0 0 3	1 0 4 5	1 0 4 4	0 9 5 1	0 9 3 4	1 0 7 5	0 9 1 5	1 0 9 4	0 9 1 4	1 0 7 4	1 0 2 3	1 0 4 3	0 9 9 3	0 9 7 3
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland													X												
Histiocytic sarcoma																					X				
Cerebrum, astrocytoma malignant		X																							
Cranial nerve, carcinoma, metastatic, Zymbal's gland																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																					X				
Leukemia mononuclear									X										X						
Squamous cell carcinoma, metastatic																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Carcinoma, metastatic, Zymbal's gland																									
Eye																+									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X		X
Carcinoma	X					X						X	X		X	X	X	X						X	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																					X				
Leukemia mononuclear									X										X						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																					X				
Sarcoma stromal, metastatic, uterus							X																		
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																					X				
Leukemia mononuclear									X										X						

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15: 2,500 ppm (continued)

Number of Days on Study	5 8 1	5 8 5	5 8 5	5 8 5	5 9 2	5 9 2	6 0 0	6 0 0	6 0 0	6 1 3	6 1 9	6 2 0	6 2 3	6 2 4	6 2 4	6 3 5	6 4 5	6 4 6	6 4 6	6 4 6	6 4 6	6 8 2	6 8 2	6 8 2	6 8 2	
Carcass ID Number	1 0 1 3	0 9 8 4	0 9 8 5	0 9 9 2	1 0 2 2	1 0 4 2	0 9 6 4	1 0 2 1	0 9 7 2	1 0 3 3	0 9 6 3	1 0 3 2	0 9 8 3	0 9 7 1	0 9 4 1	1 0 0 2	1 0 0 2	0 9 6 2	0 9 8 2	0 9 9 1	1 0 0 1	1 0 3 1	0 9 6 1	0 9 8 1	1 0 1 1	1 0 1 2	**Total Tissues/ Tumors**
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, Zymbal's gland																										1
Histiocytic sarcoma																										1
Cerebrum, astrocytoma malignant																										1
Cranial nerve, carcinoma, metastatic, Zymbal's gland																									X	1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear					X		X						X		X	X				X	X					9
Squamous cell carcinoma, metastatic		X																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																									+	1
Carcinoma, metastatic, Zymbal's gland																									X	1
Eye																										1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma										X																3
Carcinoma	X						X											X			X			X		14
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear					X								X													4
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Sarcoma stromal, metastatic, uterus																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear		X			X		X					X	X	X	X	X			X	X	X		X		X	15

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rates[a]	4/50 (8%)	4/35 (11%)	7/65 (11%)	1/50 (2%)
Effective rates[b]	4/49 (8%)	4/35 (11%)	7/64 (11%)	1/46 (2%)
Terminal rates[c]	4/40 (10%)	3/13 (23%)	2/22 (9%)	0/4 (0%)
First incidence (days)	682 (T)	506	368	620
Life table tests[d]	P=0.223	P=0.121	P=0.090	P=0.555
Logistic regression tests[d]	P=0.303N	P=0.298	P=0.385	P=0.718N
Cochran-Armitage test[d]	P=0.156N			
Fisher exact test[d]		P=0.444	P=0.436	P=0.201N
Clitoral Gland: Adenoma				
Overall rates	5/50 (10%)	5/31 (16%)	12/64 (19%)	12/50 (24%)
Effective rates	5/49 (10%)	5/31 (16%)	12/59 (20%)	12/42 (29%)
Terminal rates	4/40 (10%)	3/13 (23%)	5/22 (23%)	2/4 (50%)
First incidence (days)	666	558	432	453
Life table tests	P<0.001	P=0.074	P=0.007	P<0.001
Logistic regression tests	P=0.003	P=0.197	P=0.077	P=0.006
Cochran-Armitage test	P=0.016			
Fisher exact test		P=0.327	P=0.119	P=0.024
Clitoral Gland: Carcinoma				
Overall rates	2/50 (4%)	6/31 (19%)	12/64 (19%)	15/50 (30%)
Effective rates	2/50 (4%)	6/31 (19%)	12/64 (19%)	15/50 (30%)
Terminal rates	2/40 (5%)	1/13 (8%)	3/22 (14%)	0/4 (0%)
First incidence (days)	682 (T)	506	253	372
Life table tests	P<0.001	P=0.010	P=0.002	P<0.001
Logistic regression tests	P=0.025	P=0.057	P=0.063	P=0.005
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.032	P=0.015	P<0.001
Clitoral Gland: Adenoma or Carcinoma				
Overall rates	7/50 (14%)	11/31 (35%)	24/64 (38%)	27/50 (54%)
Effective rates	7/50 (14%)	11/31 (35%)	24/64 (38%)	27/50 (54%)
Terminal rates	6/40 (15%)	4/13 (31%)	8/22 (36%)	2/4 (50%)
First incidence (days)	666	506	253	372
Life table tests	P<0.001	P=0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.028	P=0.010	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.025	P=0.004	P<0.001
Large Intestine (Colon): Adenomatous Polyp				
Overall rates	0/50 (0%)	0/35 (0%)	3/65 (5%)	1/50 (2%)
Effective rates	0/45 (0%)	0/18 (0%)	3/35 (9%)	1/9 (11%)
Terminal rates	0/40 (0%)	0/13 (0%)	2/22 (9%)	0/4 (0%)
First incidence (days)	–[e]	–	640	646
Life table tests	P=0.017	–	P=0.053	P=0.165
Logistic regression tests	P=0.062	–	P=0.094	P=0.347
Cochran-Armitage test	P=0.034			
Fisher exact test		–	P=0.080	P=0.167

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Liver: Neoplastic Nodule				
Overall	0/50 (0%)	0/35 (0%)	2/65 (3%)	4/50 (8%)
Effective	0/46 (0%)	0/21 (0%)	2/48 (4%)	4/24 (17%)
Terminal	0/40 (0%)	0/13 (0%)	1/22 (5%)	1/4 (25%)
First incidence (days)	-	-	625	585
Life table tests	P<0.001	-	P=0.171	P=0.001
Logistic regression tests	P=0.002	-	P=0.246	P=0.016
Cochran-Armitage test	P=0.002			
Fisher exact test		-	P=0.258	P=0.012
Liver: Neoplastic Nodule or Hepatocellular Carcinoma				
Overall	0/50 (0%)	0/35 (0%)	2/65 (3%)	5/50 (10%)
Effective	0/46 (0%)	0/25 (0%)	2/49 (4%)	5/27 (19%)
Terminal	0/40 (0%)	0/13 (0%)	1/22 (5%)	1/4 (25%)
First incidence (days)	-	-	625	564
Life table tests	P<0.001	-	P=0.171	P<0.001
Logistic regression tests	P<0.001	-	P=0.246	P=0.010
Cochran-Armitage test	P<0.001			
Fisher exact test		-	P=0.263	P=0.005
Lung: Alveolar/bronchiolar Adenoma				
Overall	1/50 (2%)	2/35 (6%)	1/65 (2%)	0/50 (0%)
Effective	1/47 (2%)	2/29 (7%)	1/52 (2%)	0/29 (0%)
Terminal	1/40 (3%)	1/13 (8%)	1/22 (5%)	0/4 (0%)
First incidence (days)	682 (T)	529	682 (T)	-
Life table tests	P=0.609N	P=0.209	P=0.623	P=0.923N
Logistic regression tests	P=0.324N	P=0.399	P=0.623	P=0.923N
Cochran-Armitage test	P=0.314N			
Fisher exact test		P=0.323	P=0.727N	P=0.618N
Mammary Gland: Fibroadenoma				
Overall rates	16/50 (32%)	4/35 (11%)	16/65 (25%)	4/50 (8%)
Effective rates	16/48 (33%)	4/32 (13%)	16/54 (30%)	4/34 (12%)
Terminal rates	16/40 (40%)	1/13 (8%)	6/22 (27%)	0/4 (0%)
First incidence (days)	682 (T)	579	555	502
Life table tests	P=0.120	P=0.363N	P=0.094	P=0.214
Logistic regression tests	P=0.245N	P=0.162N	P=0.559	P=0.317N
Cochran-Armitage test	P=0.052N			
Fisher exact test		P=0.030N	P=0.425N	P=0.022N
Mammary Gland: Adenocarcinoma				
Overall rates	4/50 (8%)	3/35 (9%)	2/65 (3%)	2/50 (4%)
Effective rates	4/47 (9%)	3/32 (9%)	2/54 (4%)	2/32 (6%)
Terminal rates	3/40 (8%)	1/13 (8%)	1/22 (5%)	1/4 (25%)
First incidence (days)	664	506	625	547
Life table tests	P=0.312	P=0.308	P=0.575N	P=0.194
Logistic regression tests	P=0.400N	P=0.592	P=0.387N	P=0.641
Cochran-Armitage test	P=0.339N			
Fisher exact test		P=0.597	P=0.275N	P=0.533N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Mammary Gland: Fibroadenoma or Adenocarcinoma				
Overall rates	18/50 (36%)	7/35 (20%)	17/65 (26%)	6/50 (12%)
Effective rates	18/48 (38%)	7/32 (22%)	17/54 (31%)	6/34 (18%)
Terminal rates	17/40 (43%)	2/13 (15%)	6/22 (27%)	1/4 (25%)
First incidence (days)	664	506	555	502
Life table tests	P=0.059	P=0.504	P=0.121	P=0.045
Logistic regression tests	P=0.228N	P=0.284N	P=0.519N	P=0.439N
Cochran-Armitage test	P=0.061N			
Fisher exact test		P=0.108N	P=0.333N	P=0.043N
Oral Cavity (Tongue or Pharynx): Squamous Papilloma				
Overall rates	2/50 (4%)	3/35 (9%)	12/65 (18%)	9/50 (18%)
Effective rates	2/49 (4%)	3/35 (9%)	12/63 (19%)	9/46 (20%)
Terminal rates	2/40 (5%)	0/13 (0%)	3/22 (14%)	1/4 (25%)
First incidence (days)	682 (T)	463	583	372
Life table tests	P<0.001	P=0.213	P<0.001	P<0.001
Logistic regression tests	P=0.015	P=0.491	P=0.008	P=0.035
Cochran-Armitage test	P=0.012			
Fisher exact test		P=0.343	P=0.015	P=0.019
Oral Cavity (Tongue or Pharynx): Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	8/65 (12%)	6/50 (12%)
Effective rates	0/49 (0%)	1/35 (3%)	8/64 (13%)	6/47 (13%)
Terminal rates	0/40 (0%)	1/13 (8%)	0/22 (0%)	1/4 (25%)
First incidence (days)	-	682 (T)	432	359
Life table tests	P<0.001	P=0.277	P=0.005	P<0.001
Logistic regression tests	P=0.015	P=0.277	P=0.023	P=0.023
Cochran-Armitage test	P=0.008			
Fisher exact test		P=0.417	P=0.009	P=0.012
Oral Cavity (Tongue or Pharynx): Squamous Papilloma or Squamous Cell Carcinoma				
Overall rates	2/50 (4%)	4/35 (11%)	19/65 (29%)	15/50 (30%)
Effective rates	2/49 (4%)	4/35 (11%)	19/64 (30%)	15/47 (32%)
Terminal rates	2/40 (5%)	1/13 (8%)	3/22 (14%)	2/4 (50%)
First incidence (days)	682 (T)	463	432	359
Life table tests	P<0.001	P=0.079	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.294	P<0.001	P=0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.195	P<0.001	P<0.001
Pharynx: Squamous Papilloma				
Overall rates	0/50 (0%)	2/35 (6%)	11/65 (17%)	7/50 (14%)
Effective rates	0/49 (0%)	2/35 (6%)	11/63 (17%)	7/46 (15%)
Terminal rates	0/40 (0%)	0/13 (0%)	2/22 (9%)	0/4 (0%)
First incidence (days)	-	463	583	372
Life table tests	P<0.001	P=0.165	P<0.001	P<0.001
Logistic regression tests	P=0.012	P=0.187	P=0.002	P=0.022
Cochran-Armitage test	P=0.007			
Fisher exact test		P=0.171	P=0.001	P=0.005

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Pharynx: Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	5/65 (8%)	3/50 (6%)
Effective rates	0/49 (0%)	0/35 (0%)	5/64 (8%)	3/47 (6%)
Terminal rates	0/40 (0%)	0/13 (0%)	0/22 (0%)	0/4 (0%)
First incidence (days)	-	-	524	359
Life table tests	P=0.006	-	P=0.029	P=0.065
Logistic regression tests	P=0.112	-	P=0.074	P=0.289
Cochran-Armitage test	P=0.051			
Fisher exact test		-	P=0.054	P=0.113
Pituitary Gland (Pars Distalis): Adenoma				
Overall rates	18/49 (37%)	10/35 (29%)	19/65 (29%)	6/49 (12%)
Effective rates	18/48 (38%)	10/35 (29%)	19/61 (31%)	6/44 (14%)
Terminal rates	15/39 (38%)	5/13 (38%)	8/22 (36%)	1/4 (25%)
First incidence (days)	550	495	524	402
Life table tests	P=0.049	P=0.146	P=0.084	P=0.086
Logistic regression tests	P=0.149N	P=0.588	P=0.488N	P=0.197N
Cochran-Armitage test	P=0.010N			
Fisher exact test		P=0.271N	P=0.311N	P=0.008N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rates	18/49 (37%)	10/35 (29%)	20/65 (31%)	6/49 (12%)
Effective rates	18/48 (38%)	10/35 (29%)	20/61 (33%)	6/44 (14%)
Terminal rates	15/39 (38%)	5/13 (38%)	8/22 (36%)	1/4 (25%)
First incidence (days)	550	495	524	402
Life table tests	P=0.041	P=0.146	P=0.059	P=0.086
Logistic regression tests	P=0.161N	P=0.588	P=0.560N	P=0.197N
Cochran-Armitage test	P=0.011N			
Fisher exact test		P=0.271N	P=0.377N	P=0.008N
Skin: Keratoacanthoma				
Overall rates	2/50 (4%)	1/35 (3%)	3/65 (5%)	0/50 (0%)
Effective rates	2/46 (4%)	1/21 (5%)	3/45 (7%)	0/15 (0%)
Terminal rates	1/40 (3%)	0/13 (0%)	0/22 (0%)	0/4 (0%)
First incidence (days)	664	622	616	---
Life table tests	P=0.590	P=0.647	P=0.346	P=0.793N
Logistic regression tests	P=0.379N	P=0.694N	P=0.535	P=0.610N
Cochran-Armitage test	P=0.490N			
Fisher exact test		P=0.683	P=0.489	P=0.566N
Skin: Squamous Papilloma				
Overall rates	0/50 (0%)	2/35 (6%)	5/65 (8%)	5/50 (10%)
Effective rates	0/47 (0%)	2/28 (7%)	5/51 (10%)	5/28 (18%)
Terminal rates	0/40 (0%)	1/13 (8%)	4/22 (18%)	0/4 (0%)
First incidence (days)	-	535	673	607
Life table tests	P<0.001	P=0.087	P=0.005	P<0.001
Logistic regression tests	P=0.001	P=0.199	P=0.007	P=0.003
Cochran-Armitage test	P=0.004			
Fisher exact test		P=0.136	P=0.035	P=0.006

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Skin: Squamous Papilloma or Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	2/35 (6%)	6/65 (9%)	5/50 (10%)
Effective rates	0/47 (0%)	2/28 (7%)	6/51 (12%)	5/28 (18%)
Terminal rates	0/40 (0%)	1/13 (8%)	4/22 (18%)	0/4 (0%)
First incidence (days)	-	535	666	607
Life table tests	P<0.001	P=0.087	P=0.002	P<0.001
Logistic regression tests	P=0.001	P=0.199	P=0.004	P=0.003
Cochran-Armitage test	P=0.004			
Fisher exact test		P=0.136	P=0.017	P=0.006
Skin (Subcutaneous Tissue): Fibroma				
Overall rates	3/50 (6%)	0/35 (0%)	0/65 (0%)	0/50 (0%)
Effective rates	3/50 (6%)	0/35 (0%)	0/64 (0%)	0/50 (0%)
Terminal rates	1/40 (3%)	0/13 (0%)	0/22 (0%)	0/4 (0%)
First incidence (days)	292	-	-	-
Life table tests	P=0.105N	P=0.305N	P=0.175N	P=0.390N
Logistic regression tests	P=0.011N	P=0.111N	P=0.027N	P=0.028N
Cochran-Armitage test	P=0.038N			
Fisher exact test		P=0.198N	P=0.082N	P=0.121N
Small Intestine: Adenocarcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	1/65 (2%)	3/50 (6%)
Effective rates	0/49 (0%)	0/33 (0%)	1/56 (2%)	3/38 (8%)
Terminal rates	0/40 (0%)	0/13 (0%)	0/22 (0%)	0/4 (0%)
First incidence (days)	-	-	479	578
Life table tests	P=0.005	-	P=0.527	P=0.016
Logistic regression tests	P=0.032	-	P=0.688	P=0.075
Cochran-Armitage test	P=0.014			
Fisher exact test		-	P=0.533	P=0.080
Small Intestine (Jejunum): Adenocarcinoma				
Overall rates	0/50 (0%)	0/35 (0%)	0/65 (0%)	3/50 (6%)
Effective rates	0/46 (0%)	0/22 (0%)	0/49 (0%)	3/26 (12%)
Terminal rates	0/40 (0%)	0/13 (0%)	0/22 (0%)	0/4 (0%)
First incidence (days)	-	-	-	578
Life table tests	P=0.002	-	-	P=0.016
Logistic regression tests	P=0.008	-	-	P=0.075
Cochran-Armitage test	P=0.004			
Fisher exact test		-	-	P=0.044
Thyroid Gland (C-cell): Adenoma				
Overall rates	10/49 (20%)	3/35 (9%)	4/65 (6%)	0/50 (0%)
Effective rates	10/44 (23%)	3/17 (18%)	4/30 (13%)	0/4 (0%)
Terminal rates	8/39 (21%)	1/13 (8%)	1/22 (5%)	0/4 (0%)
First incidence (days)	659	647	659	-
Life table tests	P=0.178N	P=0.565N	P=0.348N	P=0.313N
Logistic regression tests	P=0.033N	P=0.375N	P=0.150N	P=0.165N
Cochran-Armitage test	P=0.124N			
Fisher exact test		P=0.478N	P=0.241N	P=0.379N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Thyroid Gland (C-cell): Carcinoma				
Overall rates	3/49 (6%)	0/35 (0%)	3/65 (5%)	0/50 (0%)
Effective rates	3/41 (7%)	0/13 (0%)	3/25 (12%)	0/4 (0%)
Terminal rates	2/39 (5%)	0/13 (0%)	3/22 (14%)	0/4 (0%)
First incidence (days)	669	-	682 (T)	-
Life table tests	P=0.593	P=0.375N	P=0.389	P=0.677N
Logistic regression tests	P=0.645N	P=0.322N	P=0.463	P=0.559N
Cochran-Armitage test	P=0.608			
Fisher exact test		P=0.430N	P=0.410	P=0.751N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rates	11/49 (22%)	3/35 (9%)	7/65 (11%)	0/50 (0%)
Effective rates	11/44 (25%)	3/17 (18%)	7/30 (23%)	0/4 (0%)
Terminal rates	9/39 (23%)	1/13 (8%)	4/22 (18%)	0/4 (0%)
First incidence (days)	659	647	659	-
Life table tests	P=0.352N	P=0.498N	P=0.525	P=0.282N
Logistic regression tests	P=0.100N	P=0.313N	P=0.449N	P=0.148N
Cochran-Armitage test	P=0.278N			
Fisher exact test		P=0.403N	P=0.548N	P=0.339N
Tongue: Squamous Papilloma				
Overall rates	2/50 (4%)	1/35 (3%)	1/65 (2%)	4/50 (8%)
Effective rates	2/49 (4%)	1/35 (3%)	1/63 (2%)	4/46 (9%)
Terminal rates	2/40 (5%)	0/13 (0%)	1/22 (5%)	1/4 (25%)
First incidence (days)	682 (T)	558	682 (T)	372
Life table tests	P=0.015	P=0.676	P=0.703N	P=0.022
Logistic regression tests	P=0.231	P=0.631N	P=0.703N	P=0.426
Cochran-Armitage test	P=0.179			
Fisher exact test		P=0.625N	P=0.406N	P=0.309
Tongue: Squamous Cell Carcinoma				
Overall rates	0/50 (0%)	1/35 (3%)	3/65 (5%)	3/50 (6%)
Effective rates	0/49 (0%)	1/35 (3%)	3/60 (5%)	3/42 (7%)
Terminal rates	0/40 (0%)	1/13 (8%)	0/22 (0%)	1/4 (25%)
First incidence (days)	-	682 (T)	432	585
Life table tests	P=0.003	P=0.277	P=0.118	P=0.003
Logistic regression tests	P=0.057	P=0.277	P=0.236	P=0.031
Cochran-Armitage test	P=0.059			
Fisher exact test		P=0.417	P=0.163	P=0.094
Tongue: Squamous Papilloma or Squamous Cell Carcinoma				
Overall rates	2/50 (4%)	2/35 (6%)	4/65 (6%)	7/50 (14%)
Effective rates	2/49 (4%)	2/35 (6%)	4/63 (6%)	7/46 (15%)
Terminal rates	2/40 (5%)	1/13 (8%)	1/22 (5%)	2/4 (50%)
First incidence (days)	682 (T)	558	432	372
Life table tests	P<0.001	P=0.322	P=0.209	P<0.001
Logistic regression tests	P=0.033	P=0.520	P=0.483	P=0.054
Cochran-Armitage test	P=0.030			
Fisher exact test		P=0.556	P=0.465	P=0.065

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Uterus: Adenocarcinoma				
Overall rates	1/50 (2%)	0/35 (0%)	0/65 (0%)	3/50 (6%)
Effective rates	1/46 (2%)	0/21 (0%)	0/45 (0%)	3/17 (18%)
Terminal rates	1/40 (3%)	0/13 (0%)	0/22 (0%)	0/4 (0%)
First incidence (days)	682 (T)	-	-	607
Life table tests	P=0.008	P=0.723N	P=0.619N	P=0.018
Logistic regression tests	P=0.042	P=0.723N	P=0.619N	P=0.132
Cochran-Armitage test	P=0.018			
Fisher exact test		P=0.687N	P=0.505N	P=0.057
Uterus: Adenoma or Adenocarcinoma				
Overall rates	1/50 (2%)	0/35 (0%)	1/65 (2%)	4/50 (8%)
Effective rates	1/46 (2%)	0/21 (0%)	1/45 (2%)	4/17 (24%)
Terminal rates	1/40 (3%)	0/13 (0%)	1/22 (5%)	1/4 (25%)
First incidence (days)	682 (T)	-	682 (T)	607
Life table tests	P<0.001	P=0.723N	P=0.623	P=0.001
Logistic regression tests	P=0.004	P=0.723N	P=0.623	P=0.025
Cochran-Armitage test	P=0.003			
Fisher exact test		P=0.687N	P=0.747	P=0.016
Uterus: Stromal Polyp				
Overall rates	5/50 (10%)	8/35 (23%)	12/65 (18%)	5/50 (10%)
Effective rates	5/49 (10%)	8/35 (23%)	12/60 (20%)	5/42 (12%)
Terminal rates	5/40 (13%)	4/13 (31%)	7/22 (32%)	1/4 (25%)
First incidence (days)	682 (T)	540	432	453
Life table tests	P=0.007	P=0.005	P=0.005	P=0.034
Logistic regression tests	P=0.514	P=0.056	P=0.078	P=0.630N
Cochran-Armitage test	P=0.551N			
Fisher exact test		P=0.102	P=0.127	P=0.528
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rates	6/50 (12%)	8/35 (23%)	13/65 (20%)	7/50 (14%)
Effective rates	6/49 (12%)	8/35 (23%)	13/61 (21%)	7/44 (16%)
Terminal rates	5/40 (13%)	4/13 (31%)	8/22 (36%)	1/4 (25%)
First incidence (days)	502	540	432	408
Life table tests	P=0.002	P=0.014	P=0.006	P=0.021
Logistic regression tests	P=0.453	P=0.148	P=0.115	P=0.542N
Cochran-Armitage test	P=0.442			
Fisher exact test		P=0.161	P=0.160	P=0.416
Zymbal's Gland: Adenoma				
Overall rates	0/50 (0%)	1/35 (3%)	5/65 (8%)	3/50 (6%)
Effective rates	0/49 (0%)	1/35 (3%)	5/60 (8%)	3/42 (7%)
Terminal rates	0/40 (0%)	0/13 (0%)	1/22 (5%)	0/4 (0%)
First incidence (days)	-	495	432	547
Life table tests	P=0.006	P=0.421	P=0.016	P=0.031
Logistic regression tests	P=0.118	P=0.462	P=0.067	P=0.122
Cochran-Armitage test	P=0.065			
Fisher exact test		P=0.417	P=0.047	P=0.094

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Zymbal's Gland: Carcinoma				
Overall rates	0/50 (0%)	4/35 (11%)	7/65 (11%)	14/50 (28%)
Effective rates	0/49 (0%)	4/35 (11%)	7/64 (11%)	14/50 (28%)
Terminal rates	0/40 (0%)	0/13 (0%)	1/22 (5%)	1/4 (25%)
First incidence (days)	-	465	432	296
Life table tests	P<0.001	P=0.017	P=0.007	P<0.001
Logistic regression tests	P=0.001	P=0.056	P=0.037	P=0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.027	P=0.016	P<0.001
Zymbal's Gland: Adenoma or Carcinoma				
Overall rates	0/50 (0%)	4/35 (11%)	11/65 (17%)	17/50 (34%)
Effective rates	0/49 (0%)	4/35 (11%)	11/64 (17%)	17/50 (34%)
Terminal rates	0/40 (0%)	0/13 (0%)	2/22 (9%)	1/4 (25%)
First incidence (days)	-	465	432	296
Life table tests	P<0.001	P=0.017	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.056	P=0.004	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.027	P=0.001	P<0.001
All Organs: Mononuclear Leukemia				
Overall rates	7/50 (14%)	13/35 (37%)	27/65 (42%)	15/50 (30%)
Effective rates	7/49 (14%)	13/35 (37%)	27/58 (47%)	15/42 (36%)
Terminal rates	5/40 (13%)	3/13 (23%)	11/22 (50%)	2/4 (50%)
First incidence (days)	624	463	449	453
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.006	P=0.025	P<0.001	P<0.001
Cochran-Armitage	P=0.023			
Fisher exact test		P=0.015	P<0.001	P=0.016
All Organs: Benign Tumors				
Overall rates	38/50 (76%)	27/35 (77%)	54/65 (83%)	33/50 (66%)
Effective rates	38/50 (76%)	27/35 (77%)	54/64 (84%)	33/50 (66%)
Terminal rates	30/40 (75%)	10/13 (77%)	22/22 (100%)	4/4 (100%)
First incidence (days)	292	463	368	372
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.158	P=0.471	P=0.019	P=0.259
Cochran-Armitage test	P=0.146N			
Fisher exact test		P=0.558	P=0.188	P=0.189N
All Organs: Malignant Tumors				
Overall rates	21/50 (42%)	25/35 (71%)	56/65 (86%)	48/50 (96%)
Effective rates	21/50 (42%)	25/35 (71%)	56/65 (86%)	48/50 (96%)
Terminal rates	15/40 (38%)	5/13 (38%)	17/22 (77%)	4/4 (100%)
First incidence (days)	500	463	253	296
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.076	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.007	P<0.001	P<0.001

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
All Organs: Benign and Malignant Tumors				
Overall rates	43/50 (86%)	33/35 (94%)	64/65 (98%)	49/50 (98%)
Effective rates	43/50 (86%)	33/35 (94%)	64/65 (98%)	49/50 (98%)
Terminal rates	33/40 (83%)	11/13 (85%)	22/22 (100%)	4/4 (100%)
First incidence (days)	292	463	253	296
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests	P=0.010	P=0.597	P=0.014	P=0.071
Cochran-Armitage test	P=0.009			
Fisher exact test		P=0.196	P=0.012	P=0.030

(T)Terminal sacrifice
[a] Number of neoplasm-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 neoplasm-bearing animals/effective number of animals, i.e. number of animals alive at first occurance of this neoplasm type in any of the groups
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms 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 neoplasms in animal group

TABLE B4a
Historical Incidence of Neoplasms of the Large Intestine in Female F344/N Rats Receiving No Treatment

Study	Incidence in Controls	
	Adenocarcinoma	Adenomatous Polyp or Adenocarcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]		
Decabromodiphenyl oxide	0/39	0/39
Chlorendic acid	0/49	0/49
Overall Historical Incidence[a]		
Total	0/1,601 (0%)	0/1,601 (0%)

[a] Data as of 6 March 1990, for 2-year studies

TABLE B4b
Historical Incidence of Neoplasms of the Small Intestine in Female F344/N Rats Receiving No Treatment

Study	Incidence in Controls	
	Adenocarcinoma	Adenomatous Polyp or Adenocarcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]		
Decabromodiphenyl oxide	0/49	0/49
Chlorendic acid	0/50	0/50
Overall Historical Incidence[b]		
Total	0/1,611 (0%)	0/1,611 (0%)

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies

TABLE B4c
Historical Incidence of Liver Neoplasms in Female F344/N Rats Receiving No Treatment

Study	Incidence in Controls		
	Neoplastic Nodule	Hepatocellular Carcinoma	Neoplastic Nodule or Hepatocellular Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]			
Decabromodiphenyl oxide	1/50	0/50	1/50
Chlorendic acid	1/50	0/50	1/50
Total	2/100 (2.0%)		2/100 (2.0%)
Standard deviation	0.0%		0.0%
Range	2%–2%		2%–2%
Overall Historical Incidence[b]			
Total	34/1,643 (2.1%)	3/1,643 (0.2%)	37/1,643 (2.3%)
Standard deviation	2.6%	0.6%	2.7%
Range	0%–10%	0%–2%	0%–10%

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies

TABLE B4d
Historical Incidence of Squamous Cell Neoplasms of the Oral Cavity[a] in Female F344/N Rats Receiving No Treatment

Study	Incidence in Controls	
	Squamous Papilloma	Squamous Cell Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[b]		
Decabromodiphenyl oxide	0/50	0/50
Chlorendic acid	0/50	0/50
Overall Historical Incidence[a]		
Total	1/1,643 (0.1%)	3/1,643 (0.2%)
Standard deviation	0.4%	0.6%
Range	0%–2%	0%–2%

[a] Includes oral mucosa, palate, soft palate, gums, and tongue
[b] Data as of 6 March 1990, for 2-year studies

TABLE B4e
Historical Incidence of Clitoral Gland Neoplasms in Female F344/N Rats Receiving No Treatment

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]			
Decabromodiphenyl oxide	0/50	4/50	4/50
Chlorendic acid	0/50	4/50	4/50
Total		8/100 (8.0%)	8/100 (8.0%)
Standard deviation		0.0%	0.0%
Range		8%–8%	8%–8%
Overall Historical Incidence[b]			
Total	62/1,643 (3.8%)	53/1,643 (3.2%)[c]	115/1643 (7.0%)
Standard deviation	4.4%	3.5%	4.9%
Range	0%–20%	0%–12%	0%–20%

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies
[c] Includes four adenocarcinoma NOS, three squamous cell carcinomas, and 46 carcinoma NOS.

TABLE B4f
Historical Incidence of Neoplasms of the Uterus in Female F344/N Rats Receiving No Treatment

Study	Incidence in Controls	
	Adenoma	Adenoma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]		
Decabromodiphenyl oxide	0/49	0/49
Chlorendic acid	0/50	0/50
Overall Historical Incidence[a]		
Total	1/1,632 (0.1%)	4/1,632 (0.3%)
Standard deviation	0.4%	0.7%
Range	0%–2%	0%–2%

[a] Data as of 6 March 1990, for 2-year studies

TABLE B4g
Historical Incidence of Integumentary System Basal Cell Neoplasms in Female F344/N Rats Receiving No Treatment

Study	Incidence in Controls		
	Basal Cell Tumor	Basal Cell Carcinoma	Basal Cell Tumor or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]			
Decabromodiphenyl oxide	0/50	0/50	0/50
Chlorendic acid	0/50	0/50	0/50
Overall Historical Incidence[b]			
Total	2/1,643 (0.1%)	4/1,643 (0.2%)	6/1,643 (0.4%)
Standard deviation	0.5%	0.7%	0.8%
Range	0%–2%	0%–2%	0%–2%

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies

TABLE B4h
Historical Incidence of Integumentary System Squamous Cell Neoplasms in Female F344/N Rats Receiving No Treatment

Study	Incidence in Controls		
	Squamous Papilloma	Squamous Cell Carcinoma	Squamous Papilloma or Squamous Cell Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]			
Decabromodiphenyl oxide	0/50	0/50	0/50
Chlorendic acid	0/50	0/50	0/50
Overall Historical Incidence[b]			
Total	4/1,643 (0.2%)[c]	3/1,643 (0.2%)	7/1,643 (0.4%)[c]
Standard deviation	0.7%	0.6%	0.8%
Range	0%–2%	0%–2%	0%–2%

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies
[c] Two papillomas NOS are included in the incidence data.

TABLE B4i
Historical Incidence of Sebaceous Gland Neoplasms in Female F344/N Rats Receiving No Treatment

Study	Incidence in Controls
Historical Incidence at Hazleton Laboratories America, Inc.[a]	
Decabromodiphenyl oxide	0/50
Chlorendic acid	0/50
Overall Historical Incidence[a]	
Total	0/1,643 (0%)

[a] Data as of 6 March 1990, for 2-year studies

TABLE B4j
Historical Incidence of Zymbal's Gland Neoplasms in Female F344/N Rats Receiving No Treatment

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.[a]			
Decabromodiphenyl oxide	0/50	0/50	0/50
Chlorendic acid	0/50	1/50	1/50
Total		1/100 (1.0%)	1/100 (1.0%)
Standard deviation		1.4%	1.4%
Range		0%–2%	0%–2%
Overall Historical Incidence[b]			
Total	0/1,643 (0%)	14/1,643 (0.9%)	14/1,643 (0.9%)
Standard deviation		1.5%	1.5%
Range		0%–6%	0%–6%

[a] Data as of 1 March 1989, for 2-year studies
[b] Data as of 6 March 1990, for 2-year studies

TABLE B4k
Historical Incidence of Leukemias in Female F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls
Historical Incidence at Hazleton Laboratories America, Inc.	
Decabromodiphenyl oxide	14/50
Chlorendic acid	13/50
Total	27/100 (27.0%)
Standard deviation	1.4%
Range	26%–28%
Overall Historical Incidence	
Total	324/1,643 (19.7%)
Standard deviation	8.1%
Range	6%–40%

[a] Data as of 6 March 1990, for 2-year studies

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
9-Month interim evaluation	10	0	0	10
15-Month interim evaluation	10	10	10	10
Early deaths				
Natural deaths	4	4	12	15
Moribund kills	6	18	31	31
Survivors				
Terminal sacrifice	40	13	22	4
Animals examined microscopically	50	35	65	50
Alimentary System				
Esophagus	(50)	(35)	(65)	(50)
Hyperkeratosis	1 (2%)			1 (2%)
Intestine large, cecum	(50)	(35)	(65)	(50)
Edema		1 (3%)		
Inflammation, acute			1 (2%)	1 (2%)
Necrosis, focal				1 (2%)
Ulcer				1 (2%)
Intestine large, colon	(50)	(35)	(65)	(50)
Descending colon, necrosis, focal			1 (2%)	
Intestine large, rectum	(50)	(34)	(65)	(50)
Inflammation, acute				1 (2%)
Necrosis, focal				1 (2%)
Liver	(50)	(35)	(65)	(50)
Angiectasis, focal	3 (6%)	1 (3%)	2 (3%)	
Angiectasis, multifocal	1 (2%)	2 (6%)		
Basophilic focus	34 (68%)	18 (51%)	33 (51%)	23 (46%)
Clear cell focus	7 (14%)	3 (9%)	1 (2%)	1 (2%)
Degeneration, cystic, focal		1 (3%)		1 (2%)
Eosinophilic focus	2 (4%)	2 (6%)	3 (5%)	6 (12%)
Erythrophagocytosis				1 (2%)
Fatty change			1 (2%)	
Granuloma	11 (22%)	7 (20%)	5 (8%)	2 (4%)
Hematopoietic cell proliferation	5 (10%)	5 (14%)	14 (22%)	13 (26%)
Hepatodiaphragmatic nodule	10 (20%)	1 (3%)	9 (14%)	2 (4%)
Mixed cell focus	1 (2%)			
Necrosis, coagulative			2 (3%)	
Necrosis, multifocal			3 (5%)	2 (4%)
Pigmentation			1 (2%)	
Regeneration, diffuse			1 (2%)	
Regeneration, focal			2 (3%)	3 (6%)
Regeneration, multifocal			7 (11%)	4 (8%)
Vacuolization cytoplasmic, diffuse	2 (4%)	5 (14%)	6 (9%)	6 (12%)
Vacuolization cytoplasmic, focal			2 (3%)	2 (4%)
Vacuolization cytoplasmic, multifocal	2 (4%)	1 (3%)	1 (2%)	1 (2%)
Bile duct, cyst			2 (3%)	
Bile duct, hyperplasia		1 (3%)		
Centrilobular, degeneration, diffuse		1 (3%)	3 (5%)	3 (6%)
Centrilobular, necrosis			1 (2%)	1 (2%)
Centrilobular, necrosis, diffuse	1 (2%)			2 (4%)
Mesentery	(4)	(5)	(7)	(4)
Fat, necrosis	3 (75%)	4 (80%)	6 (86%)	4 (100%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Alimentary System (continued)				
Pancreas	(49)	(35)	(65)	(49)
Atrophy	9 (18%)	4 (11%)	6 (9%)	
Pharynx		(4)	(21)	(12)
Palate, hyperkeratosis, focal			1 (5%)	2 (17%)
Palate, hyperplasia, focal		2 (50%)	2 (10%)	
Palate, hyperplasia, squamous			1 (5%)	
Palate, hyperplasia, squamous, focal			1 (5%)	
Salivary glands	(50)	(35)	(65)	(50)
Atrophy				2 (4%)
Stomach, forestomach	(50)	(35)	(65)	(50)
Acanthosis	1 (2%)	1 (3%)	4 (6%)	3 (6%)
Inflammation, chronic	1 (2%)		1 (2%)	
Ulcer, focal	1 (2%)		2 (3%)	1 (2%)
Stomach, glandular	(50)	(35)	(65)	(50)
Erosion, diffuse		1 (3%)		
Erosion, focal	1 (2%)		1 (2%)	
Erosion, multifocal	1 (2%)		1 (2%)	1 (2%)
Inflammation, chronic			1 (2%)	
Mineralization, diffuse		1 (3%)		
Ulcer, focal				1 (2%)
Tongue	(2)	(3)	(6)	(9)
Hyperkeratosis, focal				1 (11%)
Epithelium, hyperplasia, focal				1 (11%)
Tooth			(1)	(1)
Gingiva, hyperplasia, focal				1 (100%)
Cardiovascular System				
Heart	(50)	(35)	(65)	(50)
Cardiomyopathy, chronic	23 (46%)	14 (40%)	29 (45%)	20 (40%)
Mineralization, multifocal		1 (3%)		
Atrium, thrombus		1 (3%)	3 (5%)	5 (10%)
Endocardium, hyperplasia			1 (2%)	
Endocrine System				
Adrenal gland, cortex	(50)	(35)	(65)	(50)
Angiectasis	2 (4%)			
Congestion			1 (2%)	
Hyperplasia, focal	3 (6%)	1 (3%)	2 (3%)	
Hyperplasia, multifocal			2 (3%)	
Hypertrophy, focal		1 (3%)		1 (2%)
Necrosis, multifocal				1 (2%)
Vacuolization cytoplasmic, diffuse	1 (2%)	2 (6%)	1 (2%)	2 (4%)
Vacuolization cytoplasmic, focal	2 (4%)	3 (9%)	1 (2%)	
Vacuolization cytoplasmic, multifocal	1 (2%)		1 (2%)	
Adrenal gland, medulla	(50)	(35)	(65)	(50)
Hematopoietic cell proliferation				1 (2%)
Hemorrhage	1 (2%)			
Hyperplasia, focal		1 (3%)	1 (2%)	
Hyperplasia, multifocal		1 (3%)		1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Endocrine System (continued)				
Pituitary gland	(49)	(35)	(65)	(49)
Hemorrhage	1 (2%)	1 (3%)	1 (2%)	
Pars distalis, angiectasis, focal	2 (4%)	4 (11%)	6 (9%)	4 (8%)
Pars distalis, cyst	10 (20%)	9 (26%)	15 (23%)	6 (12%)
Pars distalis, cyst, multiple	1 (2%)			
Pars distalis, hyperplasia, focal	2 (4%)	2 (6%)		1 (2%)
Thyroid gland	(49)	(35)	(65)	(50)
Cyst				1 (2%)
C-cell, hyperplasia, focal	2 (4%)	5 (14%)	4 (6%)	2 (4%)
Follicular cell, hyperplasia, focal				1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(31)	(64)	(50)
Atrophy			1 (2%)	
Ectasia	10 (20%)	4 (13%)	10 (16%)	12 (24%)
Hyperplasia, focal	4 (8%)	2 (6%)	4 (6%)	3 (6%)
Hyperplasia, squamous, focal		1 (3%)	2 (3%)	1 (2%)
Hyperplasia, squamous, multifocal		1 (3%)	2 (3%)	
Inflammation, acute			1 (2%)	
Inflammation, chronic	1 (2%)			
Ovary	(50)	(35)	(64)	(50)
Cyst	6 (12%)	5 (14%)	6 (9%)	3 (6%)
Uterus	(50)	(35)	(65)	(50)
Ectasia, focal		1 (3%)	1 (2%)	
Hydrometra	1 (2%)		1 (2%)	
Prolapse			1 (2%)	
Cervix, cyst	2 (4%)			
Cervix, fibrosis	3 (6%)	1 (3%)	2 (3%)	
Endometrium, cyst	3 (6%)	3 (9%)	6 (9%)	2 (4%)
Endometrium, hyperplasia, cystic		2 (6%)	2 (3%)	
Vagina	(2)	(1)	(1)	(1)
Cyst	1 (50%)	1 (100%)	1 (100%)	
Hematopoietic System				
Bone marrow	(50)	(35)	(64)	(49)
Hyperplasia		2 (6%)	2 (3%)	2 (4%)
Hypoplasia			3 (5%)	
Myelofibrosis				2 (4%)
Necrosis, multifocal		1 (3%)		
Lymph node	(50)	(35)	(65)	(50)
Axillary, hyperplasia, lymphoid			1 (2%)	
Mediastinal, congestion				2 (4%)
Mediastinal, erythrophagocytosis	2 (4%)			
Mediastinal, hemorrhage		1 (3%)		1 (2%)
Mediastinal, hyperplasia, re cell			1 (2%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Hematopoietic System (continued)				
Lymph node, mandibular	(50)	(33)	(65)	(50)
Angiectasis			1 (2%)	
Hyperplasia, lymphoid		1 (3%)	1 (2%)	3 (6%)
Hyperplasia, re cell	1 (2%)	1 (3%)		
Lymph node, mesenteric	(50)	(34)	(65)	(50)
Angiectasis	1 (2%)		1 (2%)	
Hemorrhage				1 (2%)
Hyperplasia, lymphoid			1 (2%)	
Hyperplasia, re cell	11 (22%)	7 (21%)	5 (8%)	4 (8%)
Inflammation, acute				1 (2%)
Spleen	(50)	(35)	(65)	(50)
Atrophy	2 (4%)	3 (9%)	1 (2%)	2 (4%)
Hematopoietic cell proliferation	5 (10%)	12 (34%)	20 (31%)	18 (36%)
Hyperplasia, reticulum cell		1 (3%)	3 (5%)	
Necrosis, multifocal				1 (2%)
Pigmentation	4 (8%)	1 (3%)	5 (8%)	4 (8%)
Thymus	(47)	(31)	(63)	(45)
Congestion				1 (2%)
Cyst	1 (2%)			
Fibrosis		1 (3%)		
Integumentary System				
Mammary gland	(49)	(34)	(63)	(44)
Ectasia	1 (2%)			
Galactocele	2 (4%)		2 (3%)	2 (5%)
Hyperplasia, diffuse				1 (2%)
Hyperplasia, multifocal	1 (2%)			
Duct, ectasia	15 (31%)	6 (18%)	11 (17%)	2 (5%)
Skin	(49)	(34)	(65)	(50)
Hyperkeratosis, focal	1 (2%)			
Necrosis, focal			1 (2%)	
Epidermis, hypoplasia, focal				1 (2%)
Hair follicle, hyperplasia, basal cell, multifocal		1 (3%)		
Subcutaneous tissue, inflammation, acute				1 (2%)
Musculoskeletal System				
Bone	(13)	(4)	(12)	(7)
Sternum, osteopetrosis	13 (100%)	4 (100%)	10 (83%)	6 (86%)
Nervous System				
Brain	(50)	(35)	(65)	(50)
Compression	3 (6%)	2 (6%)	4 (6%)	2 (4%)
Hemorrhage		1 (3%)		1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15 (continued)

	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Respiratory System				
Lung	(50)	(35)	(65)	(50)
Congestion	1 (2%)	1 (3%)	1 (2%)	2 (4%)
Foreign body	1 (2%)			
Hemorrhage		1 (3%)		
Hyperplasia, lymphoid	42 (84%)	22 (63%)	41 (63%)	35 (70%)
Infiltration cellular, histiocytic	6 (12%)	4 (11%)	11 (17%)	6 (12%)
Inflammation, chronic	1 (2%)			1 (2%)
Alveolar epithelium, hyperplasia, focal	1 (2%)		2 (3%)	1 (2%)
Nose	(50)	(35)	(65)	(50)
Foreign body	1 (2%)			
Fungus		2 (6%)	2 (3%)	2 (4%)
Inflammation, acute	2 (4%)	2 (6%)	4 (6%)	1 (2%)
Metaplasia, squamous		1 (3%)	1 (2%)	
Special Senses System				
Eye		(3)	(4)	(1)
Cataract		2 (67%)	2 (50%)	
Degeneration				1 (100%)
Cornea, inflammation, chronic active			1 (25%)	
Retina, degeneration		3 (100%)	2 (50%)	
Zymbal's gland	(49)	(35)	(64)	(50)
Ectasia	1 (2%)	5 (14%)	13 (20%)	9 (18%)
Hyperplasia, focal		1 (3%)		2 (4%)
Hyperplasia, squamous, focal		2 (6%)	4 (6%)	3 (6%)
Urinary System				
Kidney	(50)	(35)	(65)	(50)
Hydronephrosis	1 (2%)		1 (2%)	1 (2%)
Infarct, acute			1 (2%)	
Infarct, chronic		1 (3%)		
Nephropathy, chronic	45 (90%)	22 (63%)	48 (74%)	29 (58%)
Cortex, cyst		1 (3%)	1 (2%)	
Renal tubule, degeneration			1 (2%)	
Renal tubule, dilatation				1 (2%)
Renal tubule, mineralization		1 (3%)		
Renal tubule, pigmentation	3 (6%)	2 (6%)	4 (6%)	2 (4%)
Transitional epithelium, hyperplasia				1 (2%)
Urinary bladder	(50)	(35)	(65)	(50)
Pigmentation, hemosiderin	1 (2%)			

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA PROTOCOL

Testing was performed as reported by Ames *et al.* (1975) with modifications as listed below and described in greater detail in Haworth *et al.* (1983) and Mortelmans *et al.* (1986). C.I. Direct Blue 15 was sent to the laboratories as coded aliquots from the Radian Corporation, Austin, TX. It was incubated with the *Salmonella typhimurium* tester strain (TA98, TA100, TA1535, or 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 was continued for an additional 48 hours.

In this assay, each test consists of triplicate plates of concurrent positive and negative controls and of at least five doses of test chemical. The high dose was limited to 10 mg/plate. Tests were repeated for all negative assays, and all positive assays were retested under the conditions that elicited the positive response.

A positive response 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 that is not dose related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A response was considered negative when no increase in revertant colonies was observed after chemical treatment.

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 Corp., Austin, TX. Overnight Difco nutrient broth cultures of *Salmonella typhimurium* strain TA1538 were used. S9 fraction was from Aroclor-induced male Fischer rat liver or noninduced female hamster liver. In the bacterial reduction system, C.I. Direct Blue 15 was reduced overnight by incubation in brain-heart infusion broth with a washed suspension of rat cecal bacteria. Extracts of the reduction mixtures were dissolved in dimethylsulfoxide (DMSO) and combined with TA1538 and rat liver S9 mix (metabolic activation enzymes and cofactors). 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 min at 37° C. The mixtures were then plated and incubated as described for the bacterial reduction system.

Each test consisted of triplicate plates of the negative control and three doses of the test chemical. The positive control, 3,3′-dimethoxybenzidine, was tested at the same molar concentrations as C.I. Direct Blue 15 for each test condition.

CHINESE HAMSTER OVARY CELL CYTOGENETICS ASSAYS

Testing was performed as reported by Galloway *et al.* (1987) and is briefly described as follows. C.I. Direct Blue 15 was sent to the laboratories as coded aliquots 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 the study chemical; the high dose was limited by toxicity and did not exceed 2.5 mg/mL.

In the SCE test without S9, CHO cells were incubated for 26 hours with the study chemical in McCoy's 5A medium supplemented with 10% fetal bovine serum, *l*-glutamine (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 containing BrdU and Colcemid, and incubation was continued for 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 study 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 incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with the study chemical for 8 hours. Colcemid was added, and incubation continued for 2 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 hours. Cells were harvested in the same manner as for treatment without S9.

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

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind, and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were scored for frequency of SCE per cell from each dose; 100 first-division metaphase cells were scored at each dose for the chromosomal aberration test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing ten 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. For aberration data, both the dose-response curve and individual dose points were statistically analyzed. For a single trial, a statistically significant ($P<0.05$) difference for one dose point and a significant trend ($P<0.015$) 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).

RESULTS

C.I. Direct Blue 15 was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 when tested in a standard preincubation protocol at concentrations of 100 to 10,000 μg/plate in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table C1) (Mortelmans *et al.*, 1986). 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 using such a reductive metabolism protocol, C.I. Direct Blue 15 was mutagenic in *Salmonella* strain TA1538 (Reid *et al.*, 1984a,b) (Table C2). Some mutagenic activity was observed in the presence of rat S9 without prior reduction, but the mutagenicity was greatly increased following reduction. The fact that the mutagenic activity of C.I. Direct Blue 15 was less than expected in the bacterial reduction system, based on the comparative activity of the dimethoxybenzidine control, can be explained by the small proportion of dye that was reduced using this system. In the FMN/hamster system, the mutagenic activity of C.I. Direct Blue 15 was more than expected and may have resulted from the formation of additional reduction products in the crude dye mixture that was tested.

In cytogenetic tests with CHO cells, C.I. Direct Blue 15 did not induce SCE at concentrations up to 750 μg/mL in the absence of S9, or 2,500 μg/mL in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Table C3) (Galloway *et al.*, 1987). No induction of chromosomal aberrations was observed in CHO cells treated with up to 2,250 μg/mL C.I. Direct Blue 15 without S9 or 2,500 μg/mL with S9 (Table C4) (Galloway *et al.*, 1987). Reductive metabolism was not used in these cytogenetic tests.

TABLE C1
Mutagenicity of C.I. Direct Blue 15 in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b]		
		-S9	+10% S9 (hamster)	+10% S9 (rat)
TA100	0	106 ± 4.7	144 ± 3.6	136 ± 8.1
	100	90 ± 4.0	116 ± 6.1	115 ± 4.3
	333	103 ± 1.8	117 ± 6.3	120 ± 2.8
	1,000	100 ± 2.1	138 ± 8.4	135 ± 5.0
	3,333	88 ± 1.9	126 ± 9.8	118 ± 2.7
	10,000	77 ± 3.2	111 ± 9.8	106 ± 4.1
Trial summary		Negative	Negative	Negative
Positive control[c]		394 ± 78.3	2,104 ± 81.4	1,230 ± 27.7
TA1535	0	3 ± 0.3	14 ± 1.9	6 ± 0.9
	100	2 ± 0.9	10 ± 1.2	6 ± 1.5
	333	3 ± 0.6	7 ± 0.3	4 ± 0.9
	1,000	3 ± 0.9	6 ± 1.7	5 ± 1.2
	3,333	1 ± 0.3	7 ± 0.9	4 ± 1.2
	10,000	1 ± 0.6	6 ± 1.5	3 ± 0.3
Trial summary		Negative	Negative	Negative
Positive control[c]		310 ± 33.8	41 ± 3.8	42 ± 4.3
TA1537	0	4 ± 0.3	6 ± 0.9	6 ± 1.2
	100	4 ± 0.9	8 ± 0.3	6 ± 1.0
	333	4 ± 1.2	7 ± 1.5	3 ± 1.2
	1,000	3 ± 1.0	3 ± 0.0	4 ± 0.3
	3,333	4 ± 0.3	4 ± 0.3	2 ± 0.3
	10,000	1 ± 0.6	4 ± 1.2	5 ± 1.2
Trial summary		Negative	Negative	Negative
Positive control[c]		72 ± 38.0	163 ± 27.8	72 ± 8.7
TA98	0	12 ± 2.2	17 ± 3.2	14 ± 3.4
	100	11 ± 1.5	16 ± 0.9	12 ± 2.0
	333	11 ± 1.3	18 ± 0.6	15 ± 1.2
	1,000	11 ± 0.9	16 ± 0.9	19 ± 0.9
	3,333	14 ± 2.5	18 ± 0.6	15 ± 0.6
	10,000	15 ± 0.9	13 ± 0.9	16 ± 1.5
Trial summary		Negative	Negative	Negative
Positive control[c]		150 ± 24.2	1,590 ± 52.8	561 ± 12.0

TABLE C1
Mutagenicity of C.I. Direct Blue 15 in *Salmonella typhimurium* (continued)

[a] Study performed at Case Western Reserve University. The detailed protocol is presented in Mortelmans *et al.* (1986). Cells and study compound or solvent (dimethylsulfoxide) 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 0 μg/plate dose.
[b] Revertants are presented as mean ± the standard error from three plates.
[c] Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE C2
Mutagenicity of C.I. Direct Blue 15 in *Salmonella typhimurium* Strain TA1538 in Bacterial and Flavin Mononucleotide (FMN) Reduction Systems

	Reductive Metabolic System/*Oxidative Metabolic System* (Revertant/plate[b])		
Dose (μM)[a]	Bacterial reduction/*rat S9*[c]	No reductive metabolism/*rat S9*	FMN reduction/*hamster S9*[d]
0.00	43	35	33
0.25	395 (709)	62 (843)	840 (235)
0.50	730 (1,073)	75 (1,203)	744 (316)
1.00	947 (1,491)	125 (1,287)	469 (366)

[a] Amount added to overnight incubation mixture, in the case of the rat cecal bacterial reduction system, or the amount added to the S9 mix using the flavin mononucleotide reduction system.
[b] Revertants are the average from at least three plates. The standard deviation was <20% of the mean for all plates. Number of revertants obtained with the positive control, 3,3′-dimethoxybenzidine at equimolar concentrations, given in parentheses after the values obtained for C.I. Direct Blue 15. For detailed protocol, see Reid *et al.* (1984a,b).
[c] 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. S9 was from noninduced female hamster livers.
[d] 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].

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by C.I. Direct Blue 15[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: Negative								
Medium		50	1,043	443	0.42	8.9	25.8	
Mitomycin-C	0.005	25	520	1,306	2.51	52.2	25.8	491.32
C.I. Direct Blue 15	250	50	1,035	435	0.42	8.7	25.8	−1.05
	500	50	1,034	423	0.40	8.5	25.8	−3.68
	750	50	1,034	439	0.42	8.8	25.8	−0.04
								P=0.574[d]
+S9[e]								
Trial 1								
Summary: Negative								
Medium		50	1,037	566	0.54	11.3	26.3	
Cyclophosphamide	1.5	50	1,039	2675	2.57	53.5	26.3	371.71
C.I. Direct Blue 15	83.3	50	1,041	545	0.52	10.9	26.3	−4.08
	833	50	1,038	658	0.63	13.2	26.3	16.41
	2,500	50	1,039	570	0.54	11.4	26.3	0.51
								P=0.060
Trial 2								
Summary: Negative								
Medium		50	1,044	468	0.44	9.4	25.5	
Cyclophosphamide	1.5	25	514	980	1.90	39.2	25.5	325.33
C.I. Direct Blue 15	2,000	50	1,033	530	0.51	10.6	25.5	14.45
	2,250	50	1,024	515	0.50	10.3	25.5	12.19
	2,500	50	1,030	488	0.47	9.8	25.5	5.69
								P=0.238

[a] Study performed at Litton Bionetics, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with study compound 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 study chemical relative to those of culture exposed to solvent. Values at least 20% above control levels are considered significant.

[c] In the absence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 to 3 hours.

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

[e] In the presence of S9, cells were incubated with study compound 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. Direct Blue 15[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[d]
Trial 1									
Harvest time: 10.5 h					Harvest time: 10.5 h				
Summary: Negative					Summary: Negative				
Medium	100	3	0.03	3.0	Medium	100	6	0.06	5.0
Mitomycin-C					Cyclophosphamide				
0.50	50	25	0.50	34.0	25	50	18	0.36	24.0
C.I. Direct Blue 15					C.I. Direct Blue 15				
1,500	100	8	0.08	7.0	2,000	100	4	0.04	4.0
1,750	100	16	0.16	10.0	2,250	100	9	0.09	7.0
2,000	100	8	0.08	6.0	2,500	100	10	0.10	9.0
				P=0.130					P=0.083
Trial 2									
Harvest time: 10.5 h					Harvest time: 10.5 h				
Summary: Negative					Summary: Negative				
Medium	100	1	0.01	1.0	Medium	100	4	0.04	4.0
Mitomycin-C					Cyclophosphamide				
0.50	50	15	0.30	26.0	50	50	35	0.70	36.0
C.I. Direct Blue 15					C.I. Direct Blue 15				
1,750	100	1	0.01	1.0	2,000	100	11	0.11	7.0
2,000	100	2	0.02	2.0	2,250	100	4	0.04	4.0
2,250	100	5	0.05	5.0	2,500	100	2	0.02	2.0
				P=0.026					P=0.840

[a] Study performed at Litton Bionetics, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with study compound 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 study compound 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 study compound or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8 to 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 aberrations tested by linear regression trend test vs. log of the dose

APPENDIX D
HEMATOLOGY, CLINICAL CHEMISTRY, AND URINALYSIS RESULTS

TABLE D1
Hematology and Clinical Chemistry for Rats in the 13-Week Drinking Water Studies of C.I. Direct Blue 15[a]

Analysis	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	30,000 ppm
Male						
n	10	10	10	10	10	3
Hematocrit (%)	47.5 ± 0.7	49.4 ± 0.5	50.6 ± 0.5	48.9 ± 0.7	45.1 ± 0.7	42.4 ± 2.2
Hemoglobin (g/dL)	16.6 ± 0.2	16.8 ± 0.1	17.2 ± 0.1	16.5 ± 0.1	16.4 ± 0.2	15.5 ± 0.6
Erythrocytes ($10^6/\mu L$)	9.17 ± 0.12	9.51 ± 0.07	9.67 ± 0.09	9.25 ± 0.09	8.54 ± 0.22	8.46 ± 0.40
Leukocytes ($10^3/\mu L$)	6.54 ± 0.26	7.09 ± 0.29	6.72 ± 0.25	6.25 ± 0.28	6.94 ± 0.46	5.73 ± 0.58
Segmented neutrophils ($10^3/\mu L$)	1.03 ± 0.10	1.29 ± 0.13	1.25 ± 0.08	0.98 ± 0.07	0.72 ± 0.07*	0.39 ± 0.11
Lymphocytes ($10^3/\mu L$)	5.40 ± 0.24	5.70 ± 0.30	5.41 ± 0.30	5.19 ± 0.31	6.12 ± 0.46	5.24 ± 0.47
Monocytes ($10^3/\mu L$)	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.10 ± 0.05
Eosinophils ($10^3/\mu L$)	0.06 ± 0.02	0.07 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.00 ± 0.00
BUN (mg/dL)	18.2 ± 0.7	17.4 ± 0.7	19.4 ± 0.5	19.9 ± 0.9	19.7 ± 0.9	19.5 ± 0.5[c]
Creatinine (mg/dL)	0.55 ± 0.02	0.56 ± 0.02	0.58 ± 0.03	0.45 ± 0.03	0.54 ± 0.02	0.55 ± 0.05[c]
ALT (IU/L)	42 ± 3	39 ± 2	45 ± 6	41 ± 4	43 ± 5	52 ± 5[c]
LDH (IU/L)	706 ± 111	694 ± 52	723 ± 79	604 ± 51	755 ± 72	834 ± 74[c]
SDH (IU/L)	9 ± 1	9 ± 1	11 ± 3	11 ± 2	16 ± 3	16 ± 2[c]

Female	0 ppm	630 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Hematocrit (%)	45.1 ± 0.8	45.2 ± 1.2	48.2 ± 0.9	48.2 ± 0.5*	48.7 ± 0.5**	47.1 ± 0.9*
Hemoglobin (g/dL)	16.4 ± 0.2	16.8 ± 0.2	17.0 ± 0.3	16.7 ± 0.2	16.7 ± 0.2	16.8 ± 0.2
Erythrocytes ($10^6/\mu L$)	8.29 ± 0.14	8.34 ± 0.21	8.89 ± 0.16*	8.93 ± 0.09*	8.96 ± 0.08**	8.63 ± 0.15*
Leukocytes ($10^3/\mu L$)	5.15 ± 0.56	4.98 ± 0.26	5.56 ± 0.36	4.89 ± 0.26	5.32 ± 0.60	5.89 ± 0.39
Segmented neutrophils ($10^3/\mu L$)	1.05 ± 0.15	0.90 ± 0.07	0.88 ± 0.10	0.75 ± 0.13	0.80 ± 0.10	0.77 ± 0.11
Lymphocytes ($10^3/\mu L$)	4.06 ± 0.45	4.01 ± 0.27	4.58 ± 0.34	4.06 ± 0.26	4.45 ± 0.54	5.08 ± 0.38*
Monocytes ($10^3/\mu L$)	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
Eosinophils ($10^3/\mu L$)	0.02 ± 0.01	0.05 ± 0.02	0.09 ± 0.01**	0.07 ± 0.02	0.04 ± 0.01	0.04 ± 0.02
BUN (mg/dL)	16.4 ± 0.4	16.9 ± 0.7	17.1 ± 0.8[b]	17.8 ± 0.4	20.2 ± 0.6**	23.2 ± 1.4**
Creatinine (mg/dL)	0.57 ± 0.03	0.52 ± 0.04	0.61 ± 0.01[b]	0.55 ± 0.03	0.64 ± 0.02	0.58 ± 0.04
ALT (IU/L)	35 ± 3[b]	30 ± 2	30 ± 2[b]	28 ± 2	28 ± 2	30 ± 2[b]
LDH (IU/L)	669 ± 73	630 ± 69	623 ± 70[b]	816 ± 83	682 ± 49	639 ± 110
SDH (IU/L)	6 ± 1[b]	6 ± 0	6 ± 1[b]	5 ± 0	6 ± 0	8 ± 1[b]

* 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. BUN=blood urea nitrogen; LDH=lactate dehydrogenase; SDH=sorbitol dehydrogenase; ALT=alanine aminotransferase.
[b] Nine rats were examined
[c] Two rats were examined

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 9-Month Interim Evaluations of C.I. Direct Blue 15[a]

Analysis	Male		Female	
	0 ppm	2,500 ppm	0 ppm	2,500 ppm
Hematocrit (%)	48.2 ± 0.7	48.0 ± 0.5	49.0 ± 0.4	42.7 ± 2.4**
Hemoglobin (g/dL)	16.6 ± 0.3	16.2 ± 0.2	16.2 ± 0.1	14.2 ± 0.9
Erythrocytes ($10^6/\mu L$)	9.32 ± 0.15	9.23 ± 0.07	8.82 ± 0.05	7.68 ± 0.48*
Mean cell volume (μ^3)	51.7 ± 0.3	51.9 ± 0.2	55.5 ± 0.2	55.8 ± 0.6
Mean cell hemoglobin (pg)	17.9 ± 0.1	17.6 ± 0.2	18.4 ± 0.1	18.5 ± 0.2
Mean cell hemoglobin concentration (g/dL)	34.2 ± 0.4	33.8 ± 0.3	33.2 ± 0.3	33.2 ± 0.4
Leukocytes ($10^3/\mu L$)	4.41 ± 0.37	4.24 ± 0.24	3.21 ± 0.14	4.13 ± 0.71
Segmented neutrophils ($10^3/\mu L$)	1.64 ± 0.15	1.71 ± 0.14	1.02 ± 0.16	1.72 ± 0.56
Lymphocytes ($10^3/\mu L$)	2.55 ± 0.25	2.35 ± 0.16	2.10 ± 0.12	2.26 ± 0.21
Monocytes ($10^3/\mu L$)	0.11 ± 0.01	0.11 ± 0.01	0.07 ± 0.01	0.12 ± 0.03
Eosinophils ($10^3/\mu L$)	0.11 ± 0.01	0.07 ± 0.02	0.02 ± 0.01	0.03 ± 0.02
Nucleated erythrocytes/100 leukocytes	1.57 ± 0.20[b]	1.33 ± 0.33[c]	2.57 ± 0.81[b]	4.75 ± 1.37[d]
BUN (mg/dL)	18.0 ± 0.5	15.6 ± 0.5**	20.7 ± 0.9	24.8 ± 2.0
Creatinine (mg/dL)	0.71 ± 0.03	0.65 ± 0.02	0.72 ± 0.03	0.68 ± 0.04
Serum glucose (mg/dL)	145 ± 4	139 ± 4	125 ± 3	131 ± 8
ALT (IU/L)	61 ± 6	30 ± 2**	36 ± 3	25 ± 2**
LDH (IU/L)	1,189 ± 64	636 ± 99**	431 ± 35	365 ± 52
SDH (IU/L)	15 ± 2	11 ± 1*	11 ± 1	8 ± 2
Serum osmolality (mOsm/kg)	309 ± 3	302 ± 2	315 ± 2	320 ± 2
TSH (ng/mL)[e]	274 ± 18	343 ± 28	299 ± 29	310 ± 53[d]
T_3 (ng/dL)[e]	74 ± 2	72 ± 2	125 ± 4	91 ± 9**[d]
T_4 (μg/dL)[e]	4 ± 0	3 ± 0*	3 ± 0	3 ± 0[f]
Urine osmolality (mOsm/kg)	2,282 ± 218[d]	3,311 ± 166**[f]	3,202 ± 315[d]	2,674 ± 378[d]
Osmolality ratio (urine/serum)	7 ± 1[d]	11 ± 1**[f]	10 ± 1[d]	8 ± 1[d]
Urine creatinine (mg/dL)	303.0 ± 44.7[d]	536.7 ± 27.0**[f]	306.4 ± 29.8[d]	243.0 ± 26.9[d]
Urine creatinine (mg/16 h)	8.67 ± 0.91[d]	7.46 ± 0.53[f]	3.54 ± 0.86[d]	3.36 ± 0.18[d]
Urine volume (mL/16 h)	3.31 ± 0.63[d]	1.35 ± 0.17**	1.31 ± 0.38[d]	1.56 ± 0.26[d]
Urine specific gravity	1.050 ± 0.004	1.060 ± 0.000*[f]	1.051 ± 0.005	1.052 ± 0.005[d]
Urine pH	6.45 ± 0.05	6.36 ± 0.09[b]	6.30 ± 0.13	6.43 ± 0.13[b]

* Significantly different ($P<0.05$) from the control group by Wilcoxon's test.
** $P<0.01$
[a] Mean ± standard error for groups of 10 animals, unless otherwise specified. BUN=blood urea nitrogen; LDH=lactate dehydrogenase; SDH=sorbitol dehydrogenase; ALT=alanine aminotransferase; TSH=thyroid-stimulating hormone.
[b] Seven rats were examined
[c] Six rats were examined
[d] Eight rats were examined
[e] T_3 and T_4 were analyzed with the Tri-Tab and Tetra-Tab Radioimmunoassay Diagnostic Kits (Nuclear Medical Laboratories). TSH analysis was performed by the method of Ridgway *et al.* (1973).
[f] Nine rats were examined

TABLE D3
Hematology, Clinical Chemistry, and Urinalysis Data for Male Rats in the 15-Month Interim Evaluations of C.I. Direct Blue 15[a]

Analysis	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Hematocrit (%)	44.3 ± 0.9	42.8 ± 1.0	42.6 ± 1.8	39.3 ± 2.1*
Hemoglobin (g/dL)	16.1 ± 0.2	16.0 ± 0.3	15.4 ± 0.6	14.7 ± 0.7
Erythrocytes ($10^6/\mu L$)	8.61 ± 0.18	8.37 ± 0.20	8.32 ± 0.40	7.80 ± 0.40
Mean cell volume (μ^3)	51.4 ± 0.2	51.1 ± 0.2	51.4 ± 0.7	50.3 ± 0.2**
Mean cell hemoglobin (pg)	18.7 ± 0.2	19.1 ± 0.2	18.6 ± 0.4	19.0 ± 0.3
Mean cell hemoglobin concentration (g/dL)	36.4 ± 0.3	37.4 ± 0.3	36.1 ± 0.6	37.6 ± 0.6
Leukocytes ($10^3/\mu L$)	5.97 ± 0.22	6.56 ± 0.28	5.84 ± 0.33	6.66 ± 0.26
Segmented neutrophils ($10^3/\mu L$)	2.36 ± 0.23	2.83 ± 0.22	2.93 ± 0.31	3.50 ± 0.34**
Lymphocytes ($10^3/\mu L$)	3.45 ± 0.29	3.63 ± 0.16	2.76 ± 0.18	3.05 ± 0.30
Monocytes ($10^3/\mu L$)	0.04 ± 0.01	0.05 ± 0.02	0.07 ± 0.03	0.08 ± 0.03
Eosinophils ($10^3/\mu L$)	0.12 ± 0.03	0.06 ± 0.01	0.07 ± 0.02	0.03 ± 0.02*
Nucleated erythrocytes/100 leukocytes	1.50 ± 0.34[b]	2.00[c]	3.00 ± 0.00[c]	1.00 ± 0.00[c]
BUN (mg/dL)	17.6 ± 0.5	17.3 ± 0.7	15.9 ± 0.4	18.2 ± 1.1
Creatinine (mg/dL)	0.63 ± 0.06[d]	0.59 ± 0.02	0.65 ± 0.02	0.62 ± 0.03
Serum glucose (mg/dL)	154 ± 5	153 ± 3	147 ± 3	156 ± 4
ALT (IU/L)	96 ± 10	80 ± 10	56 ± 6**	57 ± 8**
LDH (IU/L)	737 ± 79	454 ± 58**	604 ± 46	493 ± 53
SDH (IU/L)	33 ± 6[e]	35 ± 5	24 ± 3	28 ± 3
Serum osmolality (mOsm/kg)	320 ± 3[d]	316 ± 3	320 ± 2	322 ± 3
TSH (ng/mL)	346 ± 56[f]	292 ± 42	328 ± 30[d]	341 ± 44[d]
T_3 (ng/dL)	59 ± 2[e]	62 ± 4	61 ± 5	51 ± 4
T_4 (μg/dL)	3 ± 0[e]	3 ± 0	3 ± 0*	2 ± 0**
Urine osmolality (mOsm/kg)	1,761 ± 198	1,986 ± 271	2,307 ± 238	2,893 ± 270**
Osmolality ratio (urine/serum)	6 ± 1	6 ± 1	7 ± 1	9 ± 1**
Urine creatinine (mg/dL)	260.0 ± 26.2	266.5 ± 31.8	374.0 ± 45.0	421.0 ± 38.6**
Urine creatinine (mg/16 h)	9.01 ± 0.40	7.76 ± 0.57	8.87 ± 0.57	8.36 ± 0.80
Urine volume (mL/16 h)	3.90 ± 0.52	3.50 ± 0.62	2.75 ± 0.43	2.25 ± 0.40*
Urine specific gravity	1.050 ± 0.003	1.052 ± 0.003	1.057 ± 0.002	1.059 ± 0.001*
Urine pH	6.65 ± 0.08	7.00 ± 0.11*	7.10 ± 0.16**	7.06 ± 0.15*[e]

* 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 for groups of 10 animals, unless otherwise specified. BUN=blood urea nitrogen; LDH=lactate dehydrogenase; SDH=sorbitol dehydrogenase; ALT=alanine aminotransferase; TSH=thyroid-stimulating hormone.
[b] Six rats were examined
[c] One rat was examined
[d] Nine rats were examined
[e] Eight rats were examined
[f] Seven rats were examined

TABLE D4
Hematology, Clinical Chemistry, and Urinalysis Data for Female Rats in the 15-Month Interim Evaluations of C.I. Direct Blue 15[a]

Analysis	0 ppm	630 ppm	1,250 ppm	2,500 ppm
Hematocrit (%)	47.1 ± 0.7	45.3 ± 0.9	39.9 ± 4.0	41.7 ± 2.9
Hemoglobin (g/dL)	16.2 ± 0.2	15.8 ± 0.2	13.9 ± 1.4	14.1 ± 1.0**
Erythrocytes ($10^6/\mu L$)	8.40 ± 0.12	7.98 ± 0.17	7.07 ± 0.75	7.44 ± 0.55
Mean cell volume (μ^3)	55.9 ± 0.3	56.7 ± 0.3	57.4 ± 1.1	56.4 ± 0.7
Mean cell hemoglobin (pg)	19.3 ± 0.1	19.8 ± 0.3	20.1 ± 0.5	19.0 ± 0.2
Mean cell hemoglobin concentration (g/dL)	34.6 ± 0.3	34.9 ± 0.5	34.4 ± 0.7	33.7 ± 0.7
Leukocytes ($10^3/\mu L$)	3.61 ± 0.18	3.61 ± 0.25	5.22 ± 1.18	4.42 ± 0.52
Segmented neutrophils ($10^3/\mu L$)	1.28 ± 0.15	1.32 ± 0.12	2.46 ± 0.91	1.93 ± 0.44
Lymphocytes ($10^3/\mu L$)	2.20 ± 0.11	2.20 ± 0.17	2.63 ± 0.24	2.40 ± 0.17
Monocytes ($10^3/\mu L$)	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.04	0.05 ± 0.02
Eosinophils ($10^3/\mu L$)	0.08 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.01
Nucleated erythrocytes/100 leukocytes	2.00 ± 0.30	3.50 ± 0.70	11.22 ± 7.79[b]	1.88 ± 0.61[c]
BUN (mg/dL)	17.1 ± 0.6	16.9 ± 0.8	22.5 ± 5.6	18.5 ± 1.3
Creatinine (mg/dL)	0.79 ± 0.05	0.60 ± 0.02**	0.61 ± 0.02**	0.56 ± 0.02**
Serum glucose (mg/dL)	165 ± 7	152 ± 3	169 ± 13	147 ± 6*
ALT (IU/L)	30 ± 2	29 ± 2	59 ± 22	44 ± 17
LDH (IU/L)	349 ± 19	265 ± 30	457 ± 87	270 ± 56
SDH (IU/L)	12 ± 1	10 ± 1	21 ± 13*[b]	15 ± 4[b]
Serum osmolality (mOsm/kg)	313 ± 3	311 ± 3	318 ± 5	309 ± 3
TSH (ng/mL)	287 ± 16[c]	264 ± 15[d]	315 ± 87[e]	416 ± 57[f]
T_3 (ng/dL)	107 ± 3	112 ± 4	94 ± 7	95 ± 7
T_4 (μg/dL)	3 ± 0	3 ± 0*	2 ± 0**	2 ± 0**
Urine osmolality (mOsm/kg)	1,971 ± 230	1,402 ± 116	1,597 ± 101	1,700 ± 229
Osmolality ratio (urine/serum)	6 ± 1	5 ± 0	5 ± 0	6 ± 1
Urine creatinine (mg/dL)	196.0 ± 22.2	171.0 ± 16.0	159.5 ± 15.4	184.0 ± 18.4
Urine creatinine (mg/16 h)	5.01 ± 0.27	3.98 ± 0.39	4.56 ± 0.32	4.18 ± 0.28
Urine volume (mL/16 hr)	2.85 ± 0.33	2.65 ± 0.48	3.10 ± 0.31	2.55 ± 0.35
Urine specific gravity	1.050 ± 0.004	1.047 ± 0.004	1.048 ± 0.002	1.053 ± 0.003
Urine pH	6.80 ± 0.08	6.90 ± 0.07	7.30 ± 0.11**	7.45 ± 0.14**

* 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 for groups of 10 animals, unless otherwise specified. BUN=blood urea nitrogen; LDH=lactate dehydrogenase; SDH=sorbitol dehydrogenase; ALT=alanine aminotransferase; TSH=thyroid-stimulating hormone.
[b] Nine rats were examined
[c] Eight rats were examined
[d] Seven rats were examined
[e] Three rats were examined
[f] Six rats were examined

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

TABLE E1
Organ Weights for Rats in the 14-Day Drinking Water Studies of C.I. Direct Blue 15[a]

Organ	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	30,000 ppm
Male						
n	5	5	5	5	5	4
Necropsy body wt	207 ± 5	229 ± 4	205 ± 5	224 ± 3	220 ± 6	191 ± 5
Brain	1.76 ± 0.02	1.75 ± 0.02	1.75 ± 0.02	1.75 ± 0.01	1.74 ± 0.03	1.71 ± 0.03
Heart	0.64 ± 0.01	0.71 ± 0.02	0.65 ± 0.02	0.70 ± 0.02	0.69 ± 0.02	0.60 ± 0.02
Liver	8.73 ± 0.25	9.73 ± 0.15	8.73 ± 0.23	9.75 ± 0.19	9.79 ± 0.32	8.27 ± 0.64
Lungs	0.96 ± 0.04	0.99 ± 0.02[b]	0.93 ± 0.03[b]	0.97 ± 0.02	0.94 ± 0.04	0.85 ± 0.03
R. kidney	0.82 ± 0.00	0.85 ± 0.02	0.87 ± 0.02	0.86 ± 0.02	0.91 ± 0.02**	0.90 ± 0.04*
R. testis	1.19 ± 0.03	1.22 ± 0.03	1.25 ± 0.03	1.23 ± 0.02	1.24 ± 0.02	1.16 ± 0.02
Thymus	0.43 ± 0.05	0.43 ± 0.01	0.38 ± 0.02	0.42 ± 0.02	0.45 ± 0.02	0.38 ± 0.02
Female						
n	5	5	5	5	5	5
Necropsy body wt	150 ± 1.8	161 ± 3.3	159 ± 2.5	156 ± 2.7	157 ± 3.5	99 ± 7.0**
Brain	1.67 ± 0.03	1.66 ± 0.03	1.66 ± 0.02	1.65 ± 0.01	1.66 ± 0.02	1.60 ± 0.02
Heart	0.49 ± 0.01	0.53 ± 0.03	0.53 ± 0.02	0.51 ± 0.02	0.51 ± 0.01	0.38 ± 0.00**
Liver	5.69 ± 0.10	6.20 ± 0.16	5.77 ± 0.10	5.85 ± 0.12	6.20 ± 0.19	3.52 ± 0.32**
Lungs	0.79 ± 0.01	0.78 ± 0.02	0.77 ± 0.01	0.78 ± 0.03	0.78 ± 0.01	0.64 ± 0.01**
R. kidney	0.59 ± 0.00	0.62 ± 0.01	0.61 ± 0.02	0.61 ± 0.02	0.63 ± 0.03	0.61 ± 0.03
Thymus	0.35 ± 0.02	0.35 ± 0.01	0.37 ± 0.01	0.36 ± 0.02	0.36 ± 0.02	0.15 ± 0.03*

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Organ weights are expressed in grams (mean ± standard error).
[b] Four rats were weighed

TABLE E2
Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Drinking Water Studies of C.I. Direct Blue 15[a]

Organ	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	30,000 ppm
Male						
n	5	5	5	5	5	4
Necropsy body wt	207 ± 5	229 ± 4	205 ± 5	224 ± 3	220 ± 6	191 ± 5
Brain	8.49 ± 0.17	7.64 ± 0.11*	8.53 ± 0.14	7.82 ± 0.15	7.93 ± 0.13	8.99 ± 0.21
Heart	3.08 ± 0.03	3.11 ± 0.05	3.15 ± 0.07	3.13 ± 0.07	3.12 ± 0.10	3.14 ± 0.06
Liver	42.1 ± 0.27	42.4 ± 0.56	42.5 ± 0.61	43.6 ± 0.64	44.4 ± 0.43**	43.2 ± 2.51
Lungs	4.61 ± 0.07	4.26 ± 0.11[b]	4.54 ± 0.03[b]	4.35 ± 0.11	4.26 ± 0.14	4.45 ± 0.14
R. kidney	3.96 ± 0.10	3.71 ± 0.13	4.24 ± 0.04*	3.83 ± 0.05	4.14 ± 0.09	4.70 ± 0.13**
R. testis	5.75 ± 0.15	5.33 ± 0.07	6.11 ± 0.11	5.52 ± 0.08	5.67 ± 0.18	6.11 ± 0.10
Thymus	2.08 ± 0.21	1.86 ± 0.08	1.85 ± 0.07	1.89 ± 0.11	2.04 ± 0.10	1.98 ± 0.08
Female						
n	5	5	5	5	5	5
Necropsy body wt	150 ± 1.8	161 ± 3.3	159 ± 2.5	156 ± 2.7	157 ± 3.5	99 ± 7.0**
Brain	11.2 ± 0.10	10.3 ± 0.19*	10.5 ± 0.15	10.6 ± 0.14	10.6 ± 0.20	16.4 ± 0.99
Heart	3.25 ± 0.09	3.29 ± 0.18	3.35 ± 0.08	3.25 ± 0.10	3.26 ± 0.08	3.87 ± 0.26
Liver	38.1 ± 0.48	38.5 ± 0.83	36.4 ± 0.42	37.5 ± 0.72	39.5 ± 0.43	35.2 ± 0.89
Lungs	5.28 ± 0.15	4.86 ± 0.10	4.86 ± 0.08	5.01 ± 0.17	4.96 ± 0.06	6.57 ± 0.32
R. kidney	3.92 ± 0.07	3.87 ± 0.05	3.82 ± 0.10	3.91 ± 0.06	4.03 ± 0.11	6.15 ± 0.24**
Thymus	2.34 ± 0.12	2.20 ± 0.06	2.32 ± 0.06	2.29 ± 0.11	2.31 ± 0.12	1.45 ± 0.15**

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Organ-weight-to-body-weight ratios are expressed as mg organ weight/g body weight (mean ± standard error).
[b] Four rats were weighed

TABLE E3
Organ Weights for Rats in the 13-Week Drinking Water Studies of C.I. Direct Blue 15[a]

Organ	0 ppm	630 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	30,000 ppm
Male							
n	10	–[b]	10	10	10	10	3
Necropsy body wt	348 ± 6	–	328 ± 6	331 ± 9	327 ± 5*	321 ± 5**	229 ± 29**
Brain	1.99 ± 0.03	–	1.91 ± 0.02	1.95 ± 0.04	1.95 ± 0.02	1.96 ± 0.03	1.90 ± 0.02
Heart	0.95 ± 0.03	–	0.92 ± 0.03	0.92 ± 0.02	0.93 ± 0.02	0.90 ± 0.01	0.75 ± 0.11
Liver	9.74 ± 0.53	–	9.91 ± 0.53	10.42 ± 0.65	10.20 ± 0.52	10.21 ± 0.62	8.75 ± 1.77
Lungs	1.26 ± 0.04	–	1.25 ± 0.03	1.18 ± 0.03	1.17 ± 0.03	1.24 ± 0.04	1.10 ± 0.10
R. kidney	0.97 ± 0.03	–	0.98 ± 0.02	1.02 ± 0.03	1.12 ± 0.02**	1.15 ± 0.03**	1.40 ± 0.20**
R. testis	1.54 ± 0.05	–	1.45 ± 0.05	1.48 ± 0.04	1.40 ± 0.05**	1.43 ± 0.02**	1.45 ± 0.04
Thymus	0.29 ± 0.01	–	0.25 ± 0.01	0.26 ± 0.01	0.29 ± 0.02	0.27 ± 0.01	0.17 ± 0.05*
Female							
n	10	10	10	10	10	10	–
Necropsy body wt	195 ± 3	187 ± 3	190 ± 2	191 ± 2	190 ± 2	186 ± 2	–
Brain	1.76 ± 0.01	1.80 ± 0.02	1.80 ± 0.01	1.79 ± 0.02	1.80 ± 0.01	1.80 ± 0.02	–
Heart	0.59 ± 0.01	0.61 ± 0.01	0.59 ± 0.01	0.59 ± 0.01	0.59 ± 0.01	0.64 ± 0.02	–
Liver	4.86 ± 0.09	4.74 ± 0.06	4.81 ± 0.08	4.80 ± 0.09	4.75 ± 0.11	5.05 ± 0.13	–
Lungs	0.89 ± 0.01	0.87 ± 0.02	0.90 ± 0.03	0.90 ± 0.03	0.86 ± 0.01	0.91 ± 0.04[c]	–
R. kidney	0.60 ± 0.02	0.60 ± 0.02	0.59 ± 0.01	0.63 ± 0.01	0.67 ± 0.01**	0.75 ± 0.02**	–
Thymus	0.25 ± 0.01	0.20 ± 0.01**	0.22 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	–

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Organ weights are expressed in grams (mean ± standard error).
[b] Not applicable
[c] Eight rats were weighed

TABLE E4
Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Drinking Water Studies of C.I. Direct Blue 15[a]

Organ	0 ppm	630 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	30,000 ppm
Male							
n	10	–[b]	10	10	10	10	3
Necropsy body wt	348 ± 6	–	328 ± 6	331 ± 9	327 ± 5*	321 ± 5**	229 ± 29**
Brain	5.73 ± 0.09	–	5.84 ± 0.11	5.89 ± 0.11	5.98 ± 0.11	6.11 ± 0.08*	8.63 ± 1.25**
Heart[d]	2.74 ± 0.06	–	2.81 ± 0.06	2.80 ± 0.06	2.84 ± 0.05	2.81 ± 0.06	3.27 ± 0.18**
Liver	27.9 ± 1.18	–	30.1 ± 1.30	31.2 ± 1.27*	31.1 ± 1.30*	31.7 ± 1.56*	37.4 ± 3.46**
Lungs	3.61 ± 0.08	–	3.82 ± 0.12	3.56 ± 0.11	3.58 ± 0.08	3.88 ± 0.08*	4.87 ± 0.24**
R. kidney[d]	2.79 ± 0.06	–	2.98 ± 0.03**	3.08 ± 0.06**	3.42 ± 0.06**	3.57 ± 0.04**	6.31 ± 1.02**
R. testis	4.42 ± 0.11	–	4.43 ± 0.15	4.47 ± 0.11	4.32 ± 0.19	4.46 ± 0.06	6.51 ± 0.75*
Thymus[c]	0.83 ± 0.03	–	0.76 ± 0.03	0.79 ± 0.02	0.87 ± 0.04	0.84 ± 0.04	0.70 ± 0.16
Female							
n	10	10	10	10	10	10	–
Necropsy body wt	195 ± 3	187 ± 3	190 ± 2	191 ± 2	190 ± 2	186 ± 2	–
Brain	9.05 ± 0.14	9.60 ± 0.11*	9.48 ± 0.10*	9.37 ± 0.14*	9.51 ± 0.08*	9.67 ± 0.08**	–
Heart[c]	3.03 ± 0.04	3.27 ± 0.08	3.08 ± 0.05	3.07 ± 0.05	3.09 ± 0.06	3.45 ± 0.10*	–
Liver	25.0 ± 0.35	25.3 ± 0.18	25.3 ± 0.24	25.1 ± 0.53	25.0 ± 0.42	27.0 ± 0.52**	–
Lungs[c]	4.60 ± 0.08	4.66 ± 0.10	4.71 ± 0.16	4.73 ± 0.16	4.55 ± 0.04	4.90 ± 0.15[d]	–
R. kidney[d]	3.11 ± 0.07	3.21 ± 0.05	3.11 ± 0.05	3.32 ± 0.06*	3.51 ± 0.05**	4.02 ± 0.10**	–
Thymus[c]	1.26 ± 0.04	1.05 ± 0.04**	1.15 ± 0.03	1.20 ± 0.04	1.16 ± 0.04	1.20 ± 0.06	–

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Organ-weight-to-body-weight ratios are expressed as mg organ weight/g body weight (mean ± standard error) unless otherwise noted.
[b] Not applicable
[c] g organ weight/g body weight
[d] Eight rats were weighed

TABLE E5
Organ Weights for Rats in the 9-Month Interim Evaluations of C.I. Direct Blue 15[a]

Organ	0 ppm	2500 ppm
Male		
n	10	10
Necropsy body wt	399 ± 10	406 ± 7
Brain	2.05 ± 0.02	2.06 ± 0.02
Kidney	2.47 ± 0.08	2.58 ± 0.05
Liver	9.69 ± 0.29	10.38 ± 0.23
Female		
n	10	10
Necropsy body wt	222 ± 5	221 ± 6
Brain	1.82 ± 0.02	1.86 ± 0.02
Kidney	1.43 ± 0.03	1.51 ± 0.03*
Liver	6.15 ± 0.13	6.63 ± 0.21

* Significantly different (P≤0.05) from the control group by Wilcoxon's test
[a] Organ weights are expressed in grams (mean ± standard error).

TABLE E6
Organ-Weight-to-Body-Weight Ratios for Rats in the 9-Month Interim Evaluations of C.I. Direct Blue 15[a]

Organ	0 ppm	2500 ppm
Male		
n	10	10
Necropsy body wt	399 ± 10	406 ± 7
Brain	5.15 ± 0.10	5.07 ± 0.08
Kidney	6.20 ± 0.11	6.36 ± 0.08
Liver	24.3 ± 0.32	25.6 ± 0.32*
Female		
n	10	10
Necropsy body wt	222 ± 5	221 ± 6
Brain	8.15 ± 0.18	8.44 ± 0.25
Kidney	6.45 ± 0.11	6.83 ± 0.19
Liver	27.8 ± 0.70	30.2 ± 1.42

* Significantly different ($P \leq 0.05$) from the control group by Wilcoxon's test
[a] Organ-weight-to-body-weight ratios are expressed as mg organ weight/g body weight (mean ± standard error).

TABLE E7
Organ Weights for Rats in the 15-Month Interim Evaluations of C.I. Direct Blue 15[a]

Organ	0 ppm	630 ppm	1250 ppm	2500 ppm
Male				
n	10	10	10	10
Necropsy body wt	421 ± 6	414 ± 6	410 ± 8	402 ± 6*
Brain	2.04 ± 0.02	2.00 ± 0.03	2.13 ± 0.07	2.01 ± 0.03
Kidney	2.66 ± 0.06	2.71 ± 0.05	2.74 ± 0.05	2.83 ± 0.06
Liver	10.34 ± 0.19	10.98 ± 0.31	11.18 ± 0.31*	11.70 ± 0.28**
Female				
n	10	10	10	10
Necropsy body wt	297 ± 8	283 ± 7	269 ± 10	259 ± 8**
Brain	1.84 ± 0.02	1.83 ± 0.02	1.81 ± 0.03	1.79 ± 0.02
Kidney	1.68 ± 0.04	1.75 ± 0.04	1.70 ± 0.03	1.78 ± 0.04
Liver	6.89 ± 0.14	6.88 ± 0.15	7.32 ± 0.17	7.64 ± 0.32

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Organ weights are expressed in grams (mean ± standard error).

TABLE E8
Organ-Weight-to-Body-Weight Ratios for Rats in the 15-Month Interim Evaluations of C.I. Direct Blue 15[a]

Organ	0 ppm	630 ppm	1250 ppm	2500 ppm
Male				
n	10	10	10	10
Necropsy body wt	421 ± 6	414 ± 6	410 ± 8	402 ± 6*
Brain	4.84 ± 0.06	4.82 ± 0.06	5.23 ± 0.28	5.00 ± 0.07
Kidney	6.33 ± 0.16	6.53 ± 0.08	6.69 ± 0.11	7.04 ± 0.11**
Liver	24.6 ± 0.33	26.5 ± 0.56*	27.2 ± 0.49**	29.1 ± 0.39**
Female				
n	10	10	10	10
Necropsy body wt	297 ± 8	283 ± 7	269 ± 10	259 ± 8**
Brain	6.21 ± 0.12	6.46 ± 0.14	6.74 ± 0.27*	6.93 ± 0.18**
Kidney	5.67 ± 0.12	6.20 ± 0.12**	6.32 ± 0.32*	6.91 ± 0.23**
Liver	23.2 ± 0.38	24.4 ± 0.39	27.5 ± 1.03**	29.7 ± 1.26**

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Organ-weight-to-body-weight ratios are expressed as mg organ weight/g body weight (mean ± standard error).

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

C.I. Direct Blue 15 was obtained in two lots from the Atlantic Chemical Company and supplied to the National Toxicology Program by Dyes Environmental and Toxicology Organization, Inc., Scarsdale, NY. Because of the high salt content, the material was desalted by the analytical chemistry laboratory (Midwest Research Institute, Kansas City, MO). Lot NJ-0-62-611 was desalted in two batches and the desalted material was assigned lot numbers M110481 and M042783. Lot A03383-2 was desalted and assigned lot number M080883. The resultant salt content was reduced from approximately 25% to about 3%. Reports on purity, stability, and identity analyses performed in support of the C.I. Direct Blue 15 studies are on file at the National Institute of Environmental Health Sciences.

The three lots of the study dye, a dark blue granular powder, were identified as C.I. Direct Blue 15 by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra of C.I. Direct Blue 15 (Figures F1 and F2) (*Sadtler Standard Spectra*).

Based on the analysis of the C.I. Direct Blue 15 originally supplied, desalting was necessary to reduce the inorganic salt content to a level acceptable for bioassay. The chemical was purified by a dialysis procedure, which resulted in salt (expressed as sodium chloride) reduction of approximately 90%, based on elemental analysis. The purified chemical was then milled to particles of approximately 60 mesh.

The purity of the three desalted lots was determined by elemental analysis, Karl Fischer water analysis, potentiometric titration, spark source mass spectrometry, and chromatographic analyses. Titration of the azo groups was performed in acetic acid containing titanium (III) chloride with standardized ferric ammonium sulfate. Normal phase thin-layer chromatography (TLC) was performed on silica gel plates with two solvent systems: 1) methyl ethyl ketone: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. For lot M110481, high-performance liquid chromatography (HPLC) was performed with an Altex Ultrasphere column in a mixture of two solvents: A) 0.1% (v/v) triethanolamine in water and 10% (v/v) methanol and B) 0.1% (v/v) triethanolamine in methanol; the pH of solvent system A was adjusted to 7.2 with 55% aqueous phosphoric acid. For lots M042783 and M080883, HPLC was performed in the same manner, but solvent system A consisted of 0.1% (v/v) triethanolamine in water and 0.1% (v/v) triethanolamine in methanol (90:10). The pH levels for solvent system A in the testing of lots M047283 and M080883 were adjusted to 6.02 and 6.0, respectively, with phosphoric acid; the pH levels of solvent system B during testing of the two lots were adjusted by the addition of an amount of phosphoric acid identical to that used for each lot in system A. The solvent system ratio used for lots M110481 and M042783 ranged from 90:10 to 45:55 and from 90:10 to 60:40 for lot M080883; the flow rate was 1 mL/min. Ultraviolet detection was at 254 nm, and visible detection was at 546 nm. It was observed that the obtained chromatograms were very dependent on slight variations of mobile phase or column conditions. Therefore, concomitant HPLC analyses were necessary to obtain a reliable comparison between batches.

For lot no. M110481, elemental analysis could not be used to confirm the identity or relative purity of the major component because the sample was a complex mixture of organic and inorganic components. Elemental analysis indicated the presence of 4.2% sodium chloride and less than 0.05% sodium sulfate. Spark source mass spectrometry indicated no elemental contaminants as a result of milling. Karl Fischer analysis indicated the presence of 9.8% water. Titration of the azo groups indicated a purity of

80.3%. This value is probably enhanced by the presence of titratable impurities. Normal phase thin-layer chromatography by solvent system 1 indicated one major product spot, eight minor impurities, and one trace impurity. Solvent system 2 indicated a major spot, five minor impurities, one trace and one slight trace impurity. HPLC of this lot indicated a major peak and 13 impurities with combined peak areas of 39.2% at 254 nm and 43.5% at 546 nm relative to that of the major peak. The combined data provides a final estimate of approximately 50% by weight for the major component.

For lot no. M042783, elemental analysis could not be used to confirm the identity or relative purity of the major component because the sample was a complex mixture of organic and inorganic components. Elemental analysis indicated the presence of 2.7% sodium chloride and 0.7% sodium sulfate. Karl Fischer analysis indicated the presence of 7.1% water. Titration of the azo groups indicated a purity of 84.8%. Again, this value is probably high because of titratable impurities. Normal phase TLC indicated one major, seven minor, four trace, and eight slight trace impurities using solvent system 1. Solvent system 2 indicated one major, four minor, four trace, and six slight trace impurities. HPLC of this lot indicated a major peak and 30 impurities with combined areas of 44.7% at 254 nm and 47.0% at 546 nm relative to the major peak area. The combined data provides an estimate of approximately 50% by weight of the major component. A HPLC major peak comparison of lots M110481 and M042783 indicated a purity of 99.6% for lot no. M042783 relative to lot no. M110481.

For lot no. M080883, elemental analysis results could not be used to confirm the identity or relative purity of the major component because the sample was a complex mixture of organic and inorganic components. Elemental analysis indicated the presence of 2.2% sodium chloride and 0.14% sodium sulfate. Karl Fischer analysis indicated the presence of 2.8% water. Titration of the azo groups indicated a purity of 90.9%. Similarly, the titration is expected to give high results because of titratable impurities. Normal phase TLC indicated one major, four minor, three trace, and two slight trace impurities using solvent system 1. Solvent system 2 indicated one major, one minor, three trace, and two slight trace impurities. HPLC indicated a major peak and 36 impurities with combined areas of 64.8% at 254 nm and 61.4% at 546 nm relative to the major peak (Figure 3). Concomitant HPLC analysis with the other two lots gave almost identical cumulative peak areas for the 35 impurities of approximately 50%. The combined data provides an estimate of approximately 50% by weight of the major component. A HPLC major peak comparison of lots M080883 and M110481 indicated a purity of 98.2% for lot no. M080883 relative to lot no. M110481.

The two largest chromatographic impurities were isolated and examined by mass spectrometry (fast electron bombardment and electrospray), infrared and UV/VIS absorbance spectrophotometry and NMR spectrometry. Spectrophotometry data confirmed that the impurities were similar in structure to the major component. NMR data established that the two impurities were positional isomers of the major component, i.e. the 2,3′-methoxy and 4,5′-azo isomers, respectively. Each of these impurities was present at approximately 10% by weight of the total sample.

As a supplement to the identity and purity analyses, solvent extractions were performed to determine the concentrations of 3,3′-dimethoxybenzidine and benzidine in lot no. M042783 and lot no. M080883. HPLC indicated 826 ppm and 392 ppm 3,3′-dimethoxybenzidine in lots M042783 and M080883, respectively. Benzidine was not present at levels greater than 1 ppm in either sample.

Stability studies performed by HPLC with the system described above but with solvent A only, acetophenone as an internal standard and ultraviolet detection at 254 nm indicated that C.I. Direct Blue 15, when stored protected from light, was stable as a bulk chemical for 2 weeks at temperatures up to 60° C. During the 22-month studies, the stability of the bulk chemical was monitored by HPLC and ultraviolet/visible spectrophotometry; no degradation of the study material was seen throughout the studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared without correction for purity of the bulk chemical by mixing appropriate quantities of C.I. Direct Blue 15 with water in a volumetric flask. Chemical dissolution was verified by absorbance comparisons of filtered versus unfiltered portions of the solution. Stability studies were conducted at the analytical laboratory. The concentration of C.I. Direct Blue 15 was determined by HPLC with a μBondapak C_{18} column and a mobile phase of 67% water with 0.1% triethanolamine and 33% methanol with 0.1% triethanolamine at a flow rate of 1.2 mL/min. The pH was adjusted to 7.2 with phosphoric acid. Visible detection was at 546 nm.

C.I. Direct Blue 15 in water at the 500 ppm dose level was found to be stable for up to 21 days when stored protected from light in sealed containers at 5° C and at room temperature. Storage under simulated animal cage conditions (open to air and light) for 72 hours had no measurable effect on chemical stability.

Periodic analyses of the dose formulations of C.I. Direct Blue 15 were conducted at the study laboratory and at the analytical laboratory using ultraviolet spectroscopy. For the 14-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 twice prior to study initiation, at the study initiation, at the midpoint of the study, and again at the end of the study (Table F3). During the 22-month studies, one of every eight sets of the dose formulations was analyzed by ultraviolet spectroscopy, and animal room dose solutions were analyzed approximately every three months, after the completion of each dosing interval. Results of the dose formulation analyses for the chronic studies are presented in Table F4. Because 110 of 119 formulations were within 10% of the target concentration, it is estimated that 92% of the formulations were prepared within specifications. Results of periodic referee analysis performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table F5).

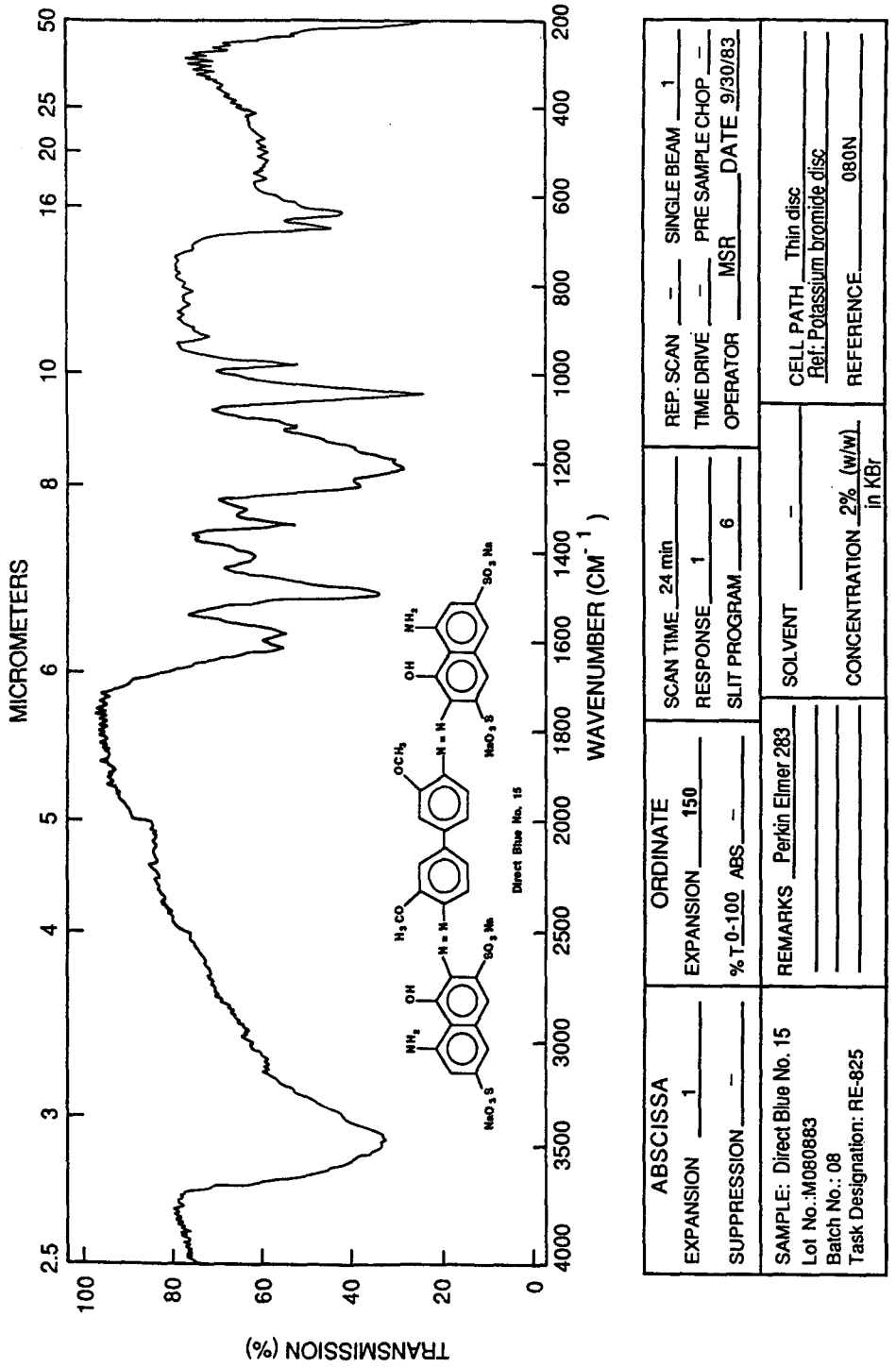

FIGURE F1
Infrared Absorption Spectrum of C.I. Direct Blue 15

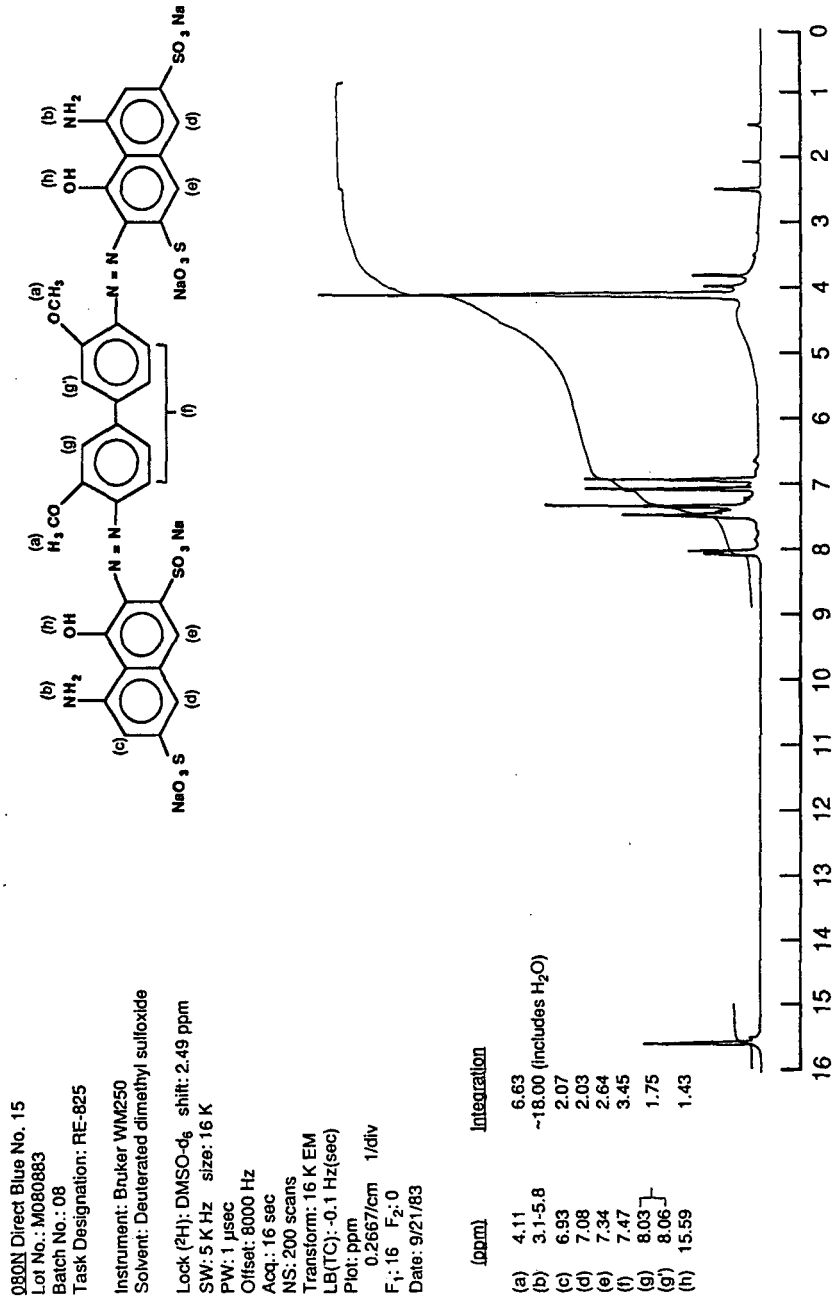

FIGURE F2
Nuclear Magnetic Resonance Spectrum of C.I. Direct Blue 15

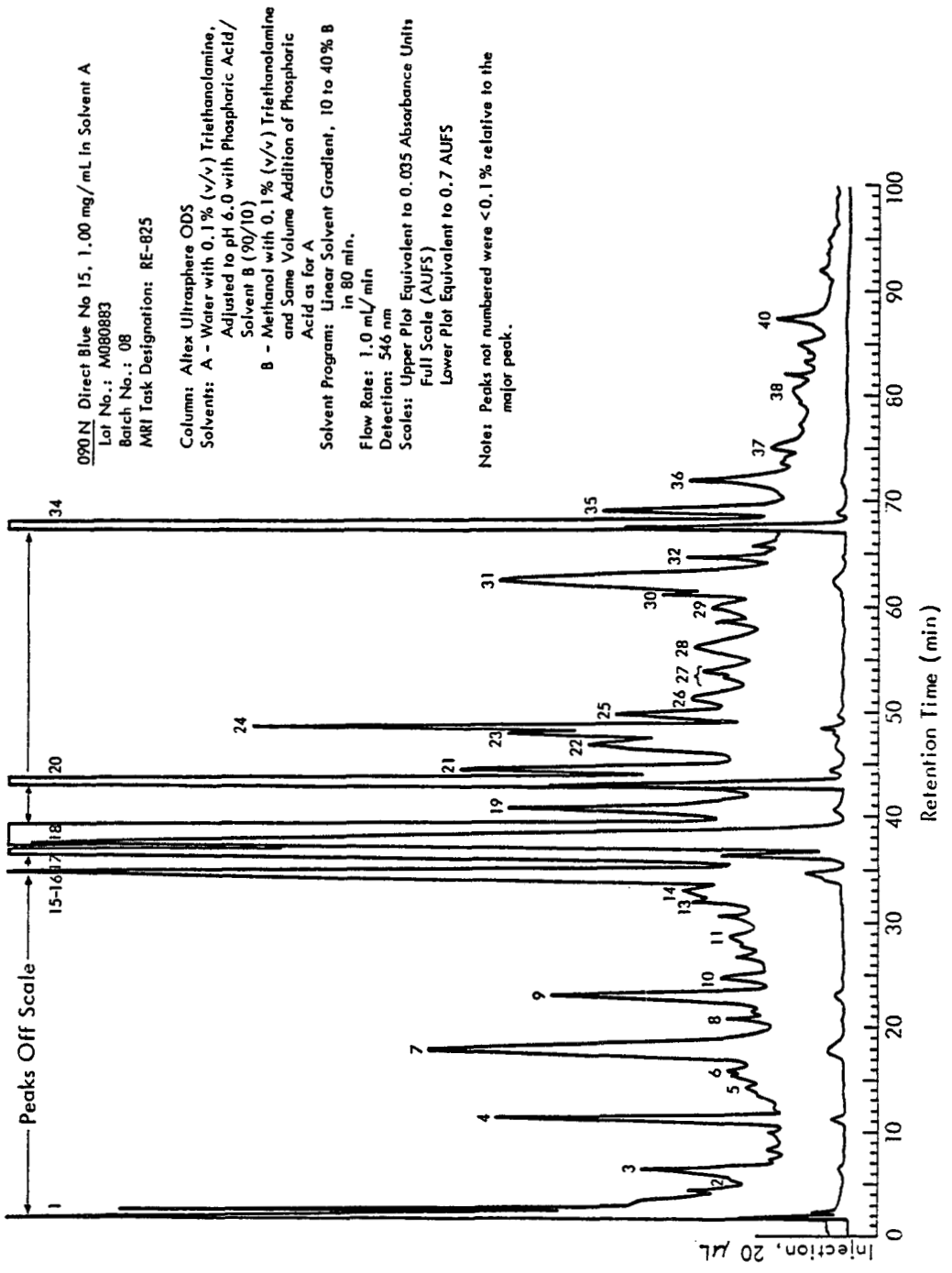

FIGURE F3
High-Performance Liquid Chromatographic Profile of C.I. Direct Blue 15 at 546 nm

TABLE F1
Preparation and Storage of Dose Formulations in the Drinking Water Studies of C.I. Direct Blue 15

14-Day Studies	13-Week Studies	22-Month Studies
Preparation		
Weighed amount of C.I. Direct Blue 15 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.	Same as 14-day studies	Weighed amount of C.I. Direct Blue 15 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		
M110481	M110481	M110481 M042783 M080883
Maximum Storage Time		
Administered on day prepared	Same as 14-day studies	1 week
Storage Conditions		
Not stored	Not stored	In the dark at room temperature

TABLE F2
Results of Analysis of Dose Formulations in the 14-Day Drinking Water Studies of C.I. Direct Blue 15

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	% Difference from Target
9 March 1982	10 March 1982	1,250	1,213	-3
		2,500	2,512	+1
		5,000	4,891	-2
		10,000	10,224	+2
		30,000	29,802	-1
25 March 1982	29 March 1982	1,250	1,324	+6
		2,500	2,580	+3
		5,000	5,317	+6
		10,000	10,512	+5
		30,000	29,270	-2

[a] Results of duplicate analysis

TABLE F3
Results of Analysis of Dose Formulations in the 13-Week Drinking Water Studies of C.I. Direct Blue 15

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	% Difference from Target
26 May 1982[b]	26 May 1982	630	670	+6
		1,250	1,350	+8
		1,250	1,340	+7
		2,500	2,530	+1
		2,500	2,020	-19[c]
		5,000	5,030	+1
		5,000	5,100	+2
		10,000	10,190	+2
		10,000	10,350	+4
		30,000	29,030	-3
27 May 1982	27 May 1982	2,500	2,510	0
1 June 1982	2 June 1982	630	650	+3
		1,250	1,290	+3
		1,250	1,300	+4
		2,500	2,550	+2
		2,500	2,590	+4
		5,000	5,130	+3
		5,000	5,320	+6
		10,000	10,290	+3
		10,000	10,490	+5
		30,000	31,560	+5
16 July 1982	16 July 1982	630	660	+5
		1,250	1,270	+2
		1,250	1,320	+6
		2,500	2,600	+4
		2,500	2,680	+7
		5,000	5,220	+4
		5,000	5,260	+5
		10,000	10,810	+8
		10,000	10,510	+5
		30,000	29,960	0
16 July 1982[d]	16 July 1982	630	660	+5
		1,250	1,250	0
		1,250	1,360	+9
		2,500	2,590	+4
		2,500	2,710	+8
		5,000	5,030	+1
		5,000	5,250	+5
		10,000	10,700	+7
		10,000	10,760	+8
		30,000	30,620	+2

TABLE F3
Results of Analysis of Dose Formulations in the 13-Week Drinking Water Studies of C.I. Direct Blue 15 (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
31 August 1982	1 September 1982	630	630	0
		1,250	1,250	0
		1,250	1,270	+2
		2,500	2,440	-2
		2,500	2,550	+2
		5,000	4,870	-3
		5,000	5,100	+2
		10,000	10,390	+4
		10,000	10,380	+4
		30,000	29,270	-2

[a] Results of duplicate analysis
[b] One week before start of study
[c] Not within tolerance. Remixed in distilled water and analyzed on 5/27/82, found to be within tolerance.
[d] Animal-room samples

TABLE F4
Results of Analysis of Dose Formulations in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	% Difference from Target
21 February 1983	23 February 1983	630	330	-48[b]
		1,250	700	-44[b]
		1,250	690	-45[b]
		2,500	2,400	-4
		2,500	2,500	0
21-24 February 1983[c]	4 March 1983	630	570	-9
		1,250	1,300	+4
		2,500	2,590	+4
24 February 1983	24 February 1983	630	640[c]	+2
		1,250	1,300[c]	+4
		1,250	1,290[c]	+3
17 March 1983	18 March 1983	630	640	+2
		1,250	1,290	+3
		1,250	1,250	0
		2,500	2,590	+4
		2,500	2,560	+2
14 April 1983	15 April 1983	630	650	+3
		1,250	1,280	+2
		1,250	1,310	+5
		2,500	2,650	+6
		2,500	2,640	+6
14 April 1983[c]	26 April 1983	630	615	-2
		1,250	1,240	-1
		2,500	2,480	-1
12 May 1983	18 May 1983	630	640	+2
		1,250	1,300	+4
		1,250	1,280	+2
		2,500	2,540	+2
		2,500	2,510	0
9 June 1983	15 June 1983	630	670	+6
		1,250	1,300	+4
		1,250	1,340	+7
		2,500	2,640	+6
		2,500	2,640	+6
7 July 1983[d]	21 July 1983	630	620	-1
		1,250	1,250	0
		2,500	2,475	-1
4 August 1983	9 August 1983	630	663	+5
		1,250	1,320	+6
		1,250	1,320	+6
		2,500	2,610	+4
		2,500	2,660	+6

TABLE F4
Results of Analysis of Dose Formulations in the 22-Month Drinking Water Studies of C.I. Direct Blue 15 (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
4 August 1983[d]	17 August 1983	630	644	+2
		1,250	1,285	+3
		2,500	2,560	+2
1 September 1983	6 September 1983	630	682	+8
		1,250	1,350	+8
		1,250	1,360	+9
		2,500	2,760	+10[b]
		2,500	2,770	+11[b]
7 September 1983	7 September 1983	2,500	2,760[c]	+10[b]
		2,500	2,760[c]	+10[b]
9 September 1983	9 September 1983	2,500	2,740[c]	+10[b]
		2,500	2,780[c]	+11[b]
15 September 1983	16 September 1983	2,500	2,540	+2
29 September 1983	3 October 1983	630	638	+1
		1,250	1,290	+3
		1,250	1,290	+3
		2,500	2,550	+2
		2,500	2,530	+1
29 September 1983[d]	14 October 1983	630	635	+1
		1,250	1,270	+2
		2,500	2,510	0
27 October 1983	1 November 1983	630	612	-3
		1,250	1,220	-2
		1,250	1,220	-2
		2,500	2,460	-2
		2,500	2,470	-1
28 November 1983	5 December 1983	630	616	-2
		1,250	1,230	-2
		1,250	1,250	0
		2,500	2,480	-1
		2,500	2,510	0
26 December 1983	28 December 1983	630	629	0
		1,250	1,250	0
		2,500	2,480	-1
26 December 1983[d]	11 February 1984	630	626	-1
		1,250	1,245	0
		2,500	2,490	0
27 December 1983[e]	28 December 1983	1,250	1,250	0

TABLE F4
Results of Analysis of Dose Formulations in the 22-Month Drinking Water Studies of C.I. Direct Blue 15 (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
19 January 1984	26 January 1984	630	628	0
		1,250	1,230	-2
		1,250	1,250	0
		2,500	2,480	-1
16 February 1984	23 February 1984	630	627	0
		1,250	1,260	+1
		1,250	1,270	+2
		2,500	2,500	0
15 March 1984	19 March 1984	630	639	+1
		1,250	1,250	0
		1,250	1,260	+1
		2,500	2,500	0
15 March 1984[d]	26 March 1984	630	626	-1
		1,250	1,210	-3
		2,500	2,440	-2
12 April 1984	17 April 1984	630	631	0
		1,250	1,260	+1
		1,250	1,270	+2
		2,500	2,520	+1
10 May 1984	16 May 1984	630	606	-4
		1,250	1,250	0
		1,250	1,250	0
		2,500	2,480	-1
7 June 1984	10 June 1984	630	641	+2
		1,250	1,260	+1
		1,250	1,370	+10
		2,500	2,500	0
7 June 1984[d]	22 June 1984	630	634	+1
		1,250	1,255	0
		2,500	2,490	0
5 July 1984	7 July 1984	630	631	0
		1,250	1,260	+1
		1,250	1,290	+3
		2,500	2,580	+3
2 August 1984	7 August 1984	630	623	-1
		1,250	1,230	-2
		1,250	1,350	+8
		2,500	2,520	+1
30 August 1984[d]	13 September 1984	630	645	+2
		1,250	1,310	+5
		2,500	2,580	+3

TABLE F4
Results of Analysis of Dose Formulations in the 22-Month Drinking Water Studies of C.I. Direct Blue 15 (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
3 September 1984	8 September 1984	630	633	+1
		1,250	1,260	+1
		1,250	1,250	0
		2,500	2,500	0
27 September 1984	28 September 1984	630	635	+1
		1,250	1,250	0
		1,250	1,270	+2
		2,500	2,540	+2
27 September 1984[d]	11 October 1984	630	624	-1
		1,250	1,230	-2
		2,500	2,480	-1
25 October 1984	27 October 1984	630	628	0
		1,250	1,250	0
		1,250	1,330	+6
		2,500	2,520	+1
26 November 1984	30 November 1984	630	619	-2
		1,250	1,250	0
		1,250	1,250	0
		2,500	2,480	-1
20 December 1984	20 December 1984	630	623	-1
		1,250	1,210	-3
		2,500	2,490	0
20 December 1984[d]	2 January 1985	630	626	-1
		1,250	1,260	+1
		2,500	2,440	-2

[a] Results of duplicate analysis
[b] Not within tolerance. Sample remixed.
[c] Analysis results of remix
[d] Animal room samples
[e] Original mix broken in transport; results of remix

TABLE F5
Results of Referee Analysis of Dose Formulations in the 22-Month Drinking Water Studies of C.I. Direct Blue 15

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory[a]	Referee Laboratory[b]
21 February 1983	630[c]	330	310
24 February 1983	630	640	629
4 August 1983	2,500	2,610	2,500
9 September 1983	2,500	–[d]	2,480
15 March 1984	1,250	1,250	1,250
27 September 1984	630	635	633

[a] Results of duplicate analysis
[b] Results of triplicate analysis
[c] Mixing error; dose formulation was not used in dosing animals
[d] Study laboratory was unable to complete the analysis due to instrumental problems

APPENDIX G
WATER AND COMPOUND CONSUMPTION IN THE 22-MONTH STUDIES

TABLE G1
Water and Compound Consumption by Male Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm		630 ppm			1,250 ppm			2,500 ppm		
Week	Water (g/day)[a]	Body Weight (g)	Water (g/day)[a]	Body Weight (g)	Dose/ Day[b]	Water (g/day)[a]	Body Weight (g)	Dose/ Day[b]	Water (g/day)[a]	Body Weight (g)	Dose/ Day[b]
4	26.7	222	23.3	227	65	23.7	223	133	23.0	215	267
5	28.8	246	26.2	248	67	25.9	240	135	22.6	235	240
8	23.2	290	23.6	285	52	23.5	283	104	21.7	279	195
9	22.6	302	23.1	297	49	21.3	294	91	20.5	289	177
13	23.6	334	23.7	323	46	22.9	330	86	20.8	324	161
14	22.5	329	24.4	340	45	22.9	337	85	21.0	333	158
16	21.5	349	19.9	350	36	22.3	337	82	18.1	339	134
17	20.5	347	19.9	356	35	20.3	343	74	17.9	344	130
21	23.3	357	22.4	363	39	22.2	356	78	20.4	356	143
25	24.3	374	24.5	376	41	24.2	372	81	21.1	370	142
29	24.8	385	22.4	382	37	23.2	388	75	20.1	385	131
33	22.7	388	23.3	386	38	23.3	392	74	18.9	387	122
37	25.1	401	21.2	391	34	22.9	395	73	21.6	397	136
41	25.4	414	29.6	410	45	25.4	407	78	22.8	408	140
45	24.8	426	25.6	409	39	24.3	410	74	22.1	405	137
49	25.8	422	24.4	412	37	25.1	411	76	21.2	405	130
53	24.0	421	24.2	413	37	23.7	415	72	20.2	405	124
57	25.3	422	32.2	417	49	23.6	410	72	22.1	405	137
61	25.2	431	34.7	395	55	26.2	414	79	25.2	405	155
65	27.6	420	23.9	410	37	22.8	413	69	22.8	398	143
69	25.8	415	24.3	406	38	23.0	405	71	23.7	397	149
73	23.6	420	22.5	410	35	23.3	403	72	41.0	393	261
77	24.7	420	24.4	406	38	28.5	401	89	26.9	387	174
81	25.2	417	39.2	400	62	27.6	404	86	34.3	382	224
85	23.6	419	23.6	408	36	25.2	400	79	32.8	385	213
89	28.1	415	23.7	391	38	24.6	382	80	28.7	365	197
93	26.3	408	28.6	377	48	56.3	386	183	59.8	334	448
97	29.3	407	34.0	386	56	30.1	371	101	45.6	331	344
4-13 Weeks:											
Mean	25.0	279	24.0	276	56	23.4	274	110	21.7	269	208
SD[c]	2.7		1.2		9	1.7		23	1.1		44
CV[d]	10.7		5.2		16.7	7.1		21.0	5.0		21.4
14-52 Weeks:											
Mean	23.7	381	23.4	380	39	23.3	377	77	20.5	376	136
SD[c]	1.7		2.8		4	1.4		4	1.6		9
CV[d]	7.3		11.8		9.6	6.2		5.2	7.8		6.9
>52 Weeks:											
Mean	25.7	418	28.0	402	44	27.9	400	88	31.9	382	214
SD[c]	1.8		5.7		9	9.3		31	11.8		97
CV[d]	7.0		20.3		21.5	33.1		35.7	36.8		45.1

[a] Grams of water consumed per animal per day; not corrected for wastage.
[b] Estimated milligrams of C.I. Direct Blue 15 consumed per day per kilogram of body weight
[c] Standard deviation of weekly mean
[d] Coefficient of variation = (standard deviation/mean) × 100

TABLE G2
Water and Compound Consumption by Female Rats in the 22-Month Drinking Water Study of C.I. Direct Blue 15

	0 ppm		630 ppm			1,250 ppm			2,500 ppm		
Week	Water (g/day)[a]	Body Weight (g)	Water (g/day)[a]	Body Weight (g)	Dose/ Day[b]	Water (g/day)[a]	Body Weight (g)	Dose/ Day[b]	Water (g/day)[a]	Body Weight (g)	Dose/ Day[b]
4	20.1	149	26.0	149	110	15.7	146	134	17.8	144	310
5	24.1	159	22.5	159	89	20.5	153	167	18.0	151	299
8	20.8	170	17.5	172	64	17.6	171	128	18.0	168	268
9	20.9	179	22.5	181	78	15.8	176	113	16.2	173	234
13	17.4	193	22.3	192	73	24.5	191	160	19.1	188	254
14	20.0	195	18.5	198	59	21.3	196	135	15.6	193	203
16	19.1	200	16.1	205	50	19.3	198	122	14.1	196	179
17	17.9	201	14.6	206	45	15.7	199	99	14.0	197	177
21	17.5	211	16.3	213	48	17.7	210	106	15.2	207	184
25	18.5	216	16.1	220	46	18.3	214	107	15.6	213	183
29	18.5	222	15.5	220	44	18.2	223	102	16.6	219	190
33	17.6	227	18.4	225	52	19.7	224	110	15.5	224	174
37	20.4	230	26.8	234	72	19.6	229	107	15.8	228	174
41	20.6	236	23.3	239	61	19.4	236	103	17.4	235	185
45	18.8	247	18.6	250	47	18.5	245	94	16.4	241	170
49	20.1	253	19.3	258	47	18.8	252	93	16.2	245	165
53	17.6	265	17.5	263	42	18.6	264	88	16.2	254	160
57	17.8	274	19.7	276	45	18.3	272	84	17.0	262	162
61	19.4	286	23.4	283	52	29.8	273	136	27.1	274	247
65	18.0	295	19.1	291	41	26.5	291	114	17.6	285	154
69	15.5	296	17.5	295	37	17.1	291	74	16.0	286	140
73	18.7	303	17.0	302	36	18.0	299	75	17.5	294	149
77	20.8	312	18.9	304	39	19.3	310	78	26.5	294	225
81	20.2	323	20.4	305	42	36.8	308	149	31.2	292	268
85	18.7	328	23.4	314	47	23.6	307	96	20.3	291	174
89	19.4	333	25.0	309	51	20.5	309	83	22.6	287	197
93	27.2	328	36.8	303	77	28.0	316	111	28.3	284	248
97	28.4	334	39.1	303	81	26.1	304	108	29.7	305	243
4-13 Weeks:											
Mean	20.6	170	22.2	171	83	18.8	167	140	17.8	163	273
SD[c]	2.4		3.0		18	3.7		23	1.1		31
CV[d]	11.5		13.6		21.4	19.6		16.2	5.9		11.5
14-52 Weeks:											
Mean	19.0	222	18.5	224	52	18.8	221	107	15.7	218	180
SD[c]	1.1		3.6		9	1.4		12	1.0		10
CV[d]	6.0		19.7		16.9	7.4		11.4	6.6		5.7
>52 Weeks:											
Mean	20.1	306	23.2	296	49	23.6	295	100	22.5	284	197
SD[c]	3.7		7.4		15	6.1		25	5.7		46
CV[d]	18.6		31.8		30.0	25.7		24.7	25.6		23.5

[a] Grams of water consumed per animal per day; not corrected for wastage.
[b] Estimated milligrams of C.I. Direct Blue 15 consumed per day per kilogram of body weight
[c] Standard deviation of weekly mean
[d] Coefficient of variation = (standard deviation/mean) × 100

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
[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	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

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

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

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.78 ± 0.84	21.3–24.9	24
Crude fat (% by weight)	5.29 ± 0.75	3.3–6.5	24
Crude fiber (% by weight)	3.45 ± 0.28	2.8–3.8	24
Ash (% by weight)	6.67 ± 0.40	6.2–7.3	24
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
Tryptophan	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	1.83–2.52	5
Linolenic	0.258 ± 0.040	0.210–0.308	5
Vitamins			
Vitamin A (IU/kg)	12,379 ± 4,800	4,100–24,000	24
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)	19.10 ± 3.78	12.0–27.0	24
Riboflavin (ppm)	7.6 ± 0.85	6.10–8.20	5
Niacin (ppm)	97.8 ± 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 (%)	1.26 ± 0.14	0.95–1.54	24
Phosphorus (%)	0.96 ± 0.06	0.87–1.10	24
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	81.7–99.40	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

Contaminants	Mean ± Standard Deviation[a]	Range	Number of Samples
Arsenic (ppm)	0.56 ± 0.18	0.17–0.77	24
Cadmium (ppm)	<0.10		24
Lead (ppm)	0.60 ± 0.23	0.33–1.32	24
Mercury (ppm)	<0.05		24
Selenium (ppm)	0.33 ± 0.06	0.21–0.42	24
Aflatoxins (ppb)	<5.0		24
Nitrate nitrogen (ppm)[b]	9.71 ± 4.98	0.10–22.0	24
Nitrite nitrogen (ppm)[b]	1.02 ± 1.68	0.10–7.20	24
BHA (ppm)[c]	2.13 ± 0.61	2.00–5.00	24
BHT (ppm)[c]	2.17 ± 1.67	1.00–4.00	24
Aerobic plate count (CFU/g)[d]	48,263 ± 38,232	7,100–130,000	24
Coliform (MPN/g)[e]	41.42 ± 102	3.00–460	24
E. coli (MPN/g)[f]	3.04 ± 0.20	<3.00–4.00	24
Total nitrosoamines (ppb)[g]	5.77 ± 5.82	1.80–30.90	24
N-Nitrosodimethylamine (ppb)[g]	4.76 ± 5.84	0.80–30.00	24
N-Nitrosopyrrolidine (ppb)[g]	1.02 ± 0.20	0.90–1.70	24
Pesticides (ppm)			
α-BHC[h]	<0.01		24
β-BHC	<0.02		24
γ-BHC	<0.01		24
δ-BHC	<0.01		24
Heptachlor	<0.01		24
Aldrin	<0.01		24
Heptachlor epoxide	<0.01		24
DDE	<0.01		24
DDD	<0.01		24
DDT	<0.01		24
HCB	<0.01		24
Mirex	<0.01		24
Methoxychlor	<0.05		24
Dieldrin	<0.01		24
Endrin	<0.01		24
Telodrin	<0.01		24
Chlordane	<0.05		24
Toxaphene	<0.1		24
Estimated PCBs	<0.2		24
Ronnel	<0.01		24
Ethion	<0.02		24
Trithion	<0.05		24
Diazinon	<0.1		24
Methyl parathion	<0.02		24
Ethyl parathion	<0.02		24
Malathion[i]	0.10 ± 0.09	0.05–0.45	24
Endosulfan I	<0.01		24
Endosulfan II	<0.01		24
Endosulfan sulfate	<0.03		24

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] Sources of contamination: alfalfa, grains, and fish meal
[c] Sources of contamination: soy oil and fish meal
[d] CFU = colony-forming unit
[e] MPN = most probable number
[f] One lot dated October contained 4 MPN/g.
[g] All values were corrected for percent recovery.
[h] BHC = hexachlorocyclohexane or benzene hexachloride
[i] Thirteen 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 these animals and the study animals are both subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

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.

Test and Method	Time of Analysis
Complement Fixation:	
RCV (rat coronavirus)	Preinitiation and termination of 13-week study; initiation of 22-month study.
Sendai	Termination of 13-week study.
ELISA:	
RCV/SDA (sialodacryoadentis virus)	6 months, 12 months, 18 months, and termination of 22-month study.
PVM, Sendai, *M. pulmonis*, *M. arthritidis*	Termination of 22-month study.
Hemagglutination Inhibition:	
PVM (pneumonia virus of mice) KRV (Kilham rat virus) H–1 (Toolan's H-1 virus)	Initiation and termination of 13-week study; initiation, 6 months, 12 months, 18 months, and termination of 22-month study.
Sendai	Initiation of 13-week study; initiation, 6 months, 12 months, and 18 months of 22-month study.

TABLE I1
Murine Virus Antibody Determinations for Rats in the 13-Week and 22-Month Drinking Water Studies of C.I. Direct Blue 15[a]

	Interval	Number of Animals	Positive Serologic Reaction for
13-Week Studies	0	10/10	none
	13 weeks	10/10	none
22-Month Studies	0	20/20	none
	6 months	10/10	RCV/SDA
		8/10	PVM
	12 months	6/10	RCV/SDA
		10/10	PVM
	18 months	7/9	RCV/SDA
		8/9	PVM
	22 months	9/10	RCV/SDA
		10/10	PVM
		2/10	KRV

[a] Blood samples taken from sentinel animals were sent to Microbiological Associates, Inc. (Bethesda, MD) for determination of antibody titers.

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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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TR No.	CHEMICAL
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
385	Methyl Bromide
386	Tetranitromethane
387	Amphetamine Sulfate
388	Ethylene Thiourea
389	Sodium Azide
390	3,3'-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
392	Chlorinated Water and Chloraminated Water
393	Sodium Fluoride
395	Probenecid
396	Monochloroacetic Acid
399	Titanocene Dichloride
401	2,4-Diaminophenol Dihydrochloride
403	Resorcinol
405	C.I. Acid Red 114
406	γ-Butyrolactone
407	C.I. Pigment Red 3
410	Naphthalene
415	Polysorbate 80
419	HC Yellow 4

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