NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 390

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 3,3′-DIMETHYLBENZIDINE DIHYDROCHLORIDE

(CAS NO. 612-82-8)

IN F344/N RATS

(DRINKING WATER STUDIES)

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

FORWORD

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 chemical 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.

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NTP TECHNICAL REPORT

ON THE

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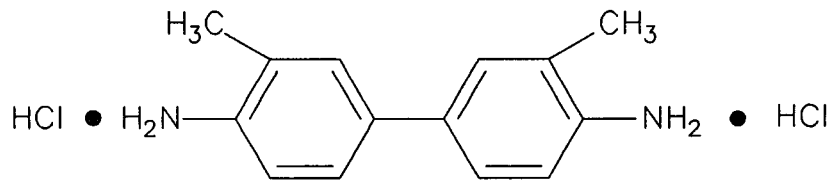
CONTENTS

ABSTRACT



3,3′-DIMETHYLBENZIDINE DIHYDROCHLORIDE

CAS No. 612-82-8

$C_{14}H_{16}N_2 \cdot 2HCl$ Molecular Weight 285.2

Synonyms: *o*-Tolidine dihydrochloride; 3,3′-Dimethylbiphenyl-4,4′-diamine dihydrochloride; 3,3′-Dimethylbiphenyl-4,4′-biphenyldiamine dihydrochloride; 4,4′-Diamino-3,3′-dimethylbiphenyl dihydrochloride

3,3′-Dimethylbenzidine dihydrochloride 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. 3,3′-Dimethylbenzidine dihydrochloride was nominated for study because of the potential for human exposure during production of bisazobiphenyl dyes and because benzidine, a structurally related chemical, is a known human carcinogen.

Toxicology and carcinogenesis studies were conducted by administering 3,3′-dimethylbenzidine dihydrochloride (approximately 99% pure) in drinking water to groups of F344/N rats of each sex for 14 days, 13 weeks, or 9 or 14 months. The 14-month exposures were planned as 24-month exposures but were terminated early because of rapidly declining animal survival, due primarily to neoplasia. These studies were performed only in rats because similar studies were being performed in mice at the National Center for Toxicological Research (NCTR). Hematologic and serum chemical analyses and thyroid hormone determinations were conducted in conjunction with the 13-week and 9-month studies. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary (CHO) cells, and *Drosophila melanogaster*.

14-Day Studies: Rats were exposed to 3,3′-dimethylbenzidine dihydrochloride in drinking water at doses ranging from 600 to 7,500 ppm. All five males and one female in the 7,500 ppm group and 1/5 males in the 5,000 ppm group died. Final mean body weights were decreased in males receiving 1,250 ppm or more and in all exposed females, and final mean body weights of animals receiving 2,500 ppm or more were lower than initial weights. Water consumption decreased with increasing

chemical concentration. Compound-related effects observed in rats receiving 5,000 ppm or more included minimal to slight hepatocellular necrosis, accumulation of brown pigment (presumably bile) in individual hepatocytes, increased severity of nephropathy relative to controls, and severe lymphocytic atrophy of the thymus. Treated animals also showed an increased severity of atrophy of the bone marrow relative to controls, varying degrees of lymphocytic atrophy of the mandibular and mesenteric lymph nodes and spleen, increased vacuolization and necrosis of cells of the adrenal cortex, focal acinar cell degeneration in the pancreas, and, in males, increased immature sperm forms in the testis and epididymis.

13-Week Studies: 3,3′-Dimethylbenzidine dihydrochloride was administered in drinking water at doses of 300, 500, 1,000, 2,000, and 4,000 ppm. All rats receiving 4,000 ppm and 4/10 males and 1/10 females receiving 2,000 ppm died before the end of the studies. Depressions in final mean body weight relative to controls ranged from 12% to 48% for males and from 9% to 42% for females. Water consumption decreased with increasing dose. At compound concentrations of 300 to 2,000 ppm, mean water consumption was 29% to 83% of control values. Compound-related effects included an increase in the severity of nephropathy relative to controls; hepatocellular necrosis and accumulation of brown pigment (presumably bile) in sinusoidal lining cells; lymphocytic atrophy of the thymus, spleen, and mandibular and mesenteric lymph nodes; atrophy of the bone marrow in the higher-dose groups; degeneration of pancreatic acinar cells; and, in males, immature sperm forms in the testis and epididymis. Decreases in serum triiodothyronine (T_3) values were observed in exposed females, and decreases in mean thyroxin (T_4) concentrations in exposed males and females; no significant changes were observed in thyroid stimulating hormone (TSH) levels in exposed rats.

Based on the decreased survival, reductions in water consumption and body weight gain, and chemical-induced hepatocellular and renal lesions observed in the 13-week studies, the doses selected for the 9- and 14-month drinking water studies of 3,3′-dimethylbenzidine dihydrochloride were 0, 30, 70, and 150 ppm. Seventy rats of each sex were used in the control group, 45 in the low-dose group, 75 in the mid-dose group, and 70 in the high-dose group.

9-Month Studies: Ten rats of each sex in the control and 150 ppm dose groups were evaluated after 9 months. Chemical-related effects observed in exposed animals included alveolar/bronchiolar carcinoma in one male, basal cell carcinoma of the skin in one male, a squamous cell carcinoma of the oral cavity in one female, preputial gland carcinoma in two males, clitoral gland carcinoma in three females, adenocarcinoma of the small intestine in two males, Zymbal's gland carcinoma in two males and three females, hepatocellular carcinoma in two males, and adenomatous polyps of the large intestine in three males. Other effects seen in dosed rats included focal cellular alteration in the liver, lymphoid atrophy in the spleen, and increased severity of nephropathy relative to controls. An increase in serum T_3 values was observed in exposed males, and a decrease in mean T_4 concentrations in exposed males and females. TSH concentrations were increased in exposed male and female rats.

Body Weights and Survival in the 14-Month Studies: The average amount of 3,3′-dimethylbenzidine dihydrochloride consumed per day was approximately 1.8, 4.0, or 11.2 mg/kg for low-, mid-, or high-dose male rats and 3.0, 6.9, or 12.9 mg/kg for low-, mid-, or high-dose female rats. The mean body weight of high-dose males was about 85% of the control value by week 28. By the end of the study, mean body weights of low-, mid-, and high-dose males were 97%, 92%, and 70% of the control values, respectively. Mean body weights of high- and mid-dose females were about 85% of control values at week 32 and week 44, respectively. At the end of the study, mean body weights of exposed females were about 94%, 81%, and 74% of control values for low-, mid-, and high-dose groups, respectively. Because of extensive neoplasia, many exposed males and females were dying or were sacrificed moribund in the first year, and all high-dose males died by week 55. The studies were terminated at weeks 60 to 61, at which time the group survivals were male: control, 60/60; low dose, 41/45; mid dose, 50/75; high dose, 0/60; female: 59/60; 39/45; 32/75; 10/60.

Nonneoplastic Effects in the 14-Month Studies: Increases in nonneoplastic lesions in dosed rats included cystic degeneration and foci of cellular alteration in the liver; exacerbation of nephropathy; and focal or multifocal hyperplasia of the Zymbal's gland, preputial and clitoral glands, and alveolar epithelium.

Neoplastic Effects in the 14-Month Studies: Neoplasms were observed in exposed rats at many sites: skin, Zymbal's gland, preputial and clitoral glands, liver, oral cavity, small and large intestine, mammary gland, lung, brain, and mesothelium. The incidence of these neoplastic effects in male and female rats is summarized in the table at the end of this section.

Genetic Toxicology: 3,3′-Dimethylbenzidine dihydrochloride was mutagenic in *Salmonella typhimurium* strain TA98 with exogenous metabolic activation; it was not mutagenic in strains TA100, TA1535, or TA97 with or without activation. 3,3′-Dimethylbenzidine dihydrochloride induced sister-chromatid exchanges (CHO) and chromosomal aberrations in CHO cells in the absence of exogenous metabolic activation; these effects were not evident in tests with S9 activation. Sex-linked recessive lethal mutations were induced in germ cells of adult male *Drosophilia melanogaster* administered 3,3′-dimethyl-benzidine dihydrochloride in feed or by injection. No reciprocal translocations occurred in *D. melanogaster* germ cells following exposure to 3,3′-dimethylbenzidine dihydrochloride.

Conclusions: Under the conditions of these 14-month drinking water studies, there was *clear evidence of carcinogenic activity** of 3,3′-dimethylbenzidine dihydrochloride for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity, small and large intestine, lung, and mesothelium. Increased incidences of neoplasms of the brain may have been related to chemical administration. There was *clear evidence of carcinogenic activity* for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity, small and large intestine, mammary gland, and lung. Increased incidences of neoplasms of the brain and mononuclear cell leukemia may have been related to chemical administration.

* 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 appears on page 11.

Summary of the 14-Month Drinking Water Studies and Genetic Toxicology of 3,3′-Dimethylbenzidine Dihydrochloride

Male F344/N Rats	Female F344/N Rats
Drinking water concentrations	
0, 30, 70, or 150 ppm 3,3′-dimethylbenzidine dihydrochloride	0, 30, 70, or 150 ppm 3,3′-dimethylbenzidine dihydrochloride
Body weights	
Exposed groups lower than controls	Exposed groups lower than controls
2-Year survival rates	
60/60, 41/45, 50/75, 0/60[a]	59/60, 39/45, 32/75, 10/60[a]
Nonneoplastic effects	
Preputial gland: hyperplasia	Clitoral gland: hyperplasia
Liver: cystic degeneration, focal cellular alterations	Liver: cystic degeneration, focal cellular alterations
Lung: hyperplasia	Lung: hyperplasia
Zymbal's gland: hyperplasia	Zymbal's gland: hyperplasia
Neoplastic effects	
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, 2/45, 2/75, 1/60
Uncertain findings	Mononuclear cell leukemia: 1/60, 3/45, 6/75, 4/60
Brain neoplasms: 0/60, 0/45, 1/75, 2/60	
Level of evidence of carcinogenic activity	
Clear evidence	Clear evidence

Genetic toxicology

Salmonella typhimurium	
Gene mutation:	Positive with S9 in strain TA98; Negative with or without S9 in strains TA100, TA1535, or TA97
Sister chromatid exchanges	
Chinese hamster ovary cells *in vitro*:	Positive without S9
Chromosomal aberrations	
Chinese hamster overy cells *in vitro*:	Positive without S9
Sex-linked recessive lethal mutations	
Drosophila melanogaster in vitro:	Positive administered by injection or in feed
Reciprocal translocations	
Drosophila melanogaster in vitro:	Negative administered in feed

[a] Reduced survival in exposed groups was due to neoplasia.

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 chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

- **Equivocal Evidence** of carcinogenic activity describes studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.

- **No Evidence** of carcinogenic activity describes studies that are interpreted as showing no chemically 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 3,3′-dimethylbenzidine dihydrochloride on April 25, 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 April 25, 1990, the draft Technical Report on the toxicology and carcinogenesis studies of 3,3′-dimethylbenzidine dihydrochloride received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. D. L. Morgan, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (*clear evidence of carcinogenic activity* for male and female rats). Dr. Morgan explained that the studies were intended to last 24 months but were terminated after 14 months because of rapidly declining survival of exposed animals, due primarily to neoplasia.

Dr. McKnight, a principal reviewer, agreed with the conclusions.

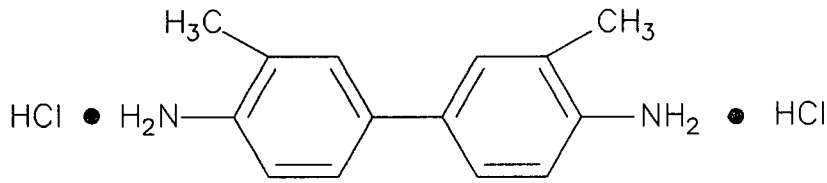
Dr. Zeise, the second principal reviewer, agreed with the conclusions with the exceptions that she felt (1) the marginally increased incidences of benign pheochromocytomas of the adrenal gland medulla in male rats may have been treatment-related, and (2) the marginally increased incidences of mononuclear cell leukemias in female rats may have been treatment-related. Dr. Morgan said pheochromocytomas were commonly occurring tumors in male rats and there was not an increased incidence of hyperplasias. With regard to leukemia, he noted that the study was terminated at 14 months and most leukemias develop after this time. Thus, the rats were not at risk long enough to determine if leukemia was treatment related. Dr. Zeise thought that liver neoplasia in the rat should be reported according to the current classification system, whereby the diagnosis of "neoplastic nodule" is given as either "hepatocellular adenoma" or "hyperplasia." Dr. Morgan explained that "neoplastic nodule" was the accepted terminology when the slides for these liver lesions were read.

Dr. Davis, the third principal reviewer, agreed with the conclusions.

Dr. William Allaben, National Center for Toxicologic Research (NCTR), reported on the 2-year studies of 3,3′-dimethylbenzidine dihydrochloride administrered to BALB/c mice at dose levels ranging from 5 to 140 ppm in drinking water. The only lesions of consequence in these studies were fatal alveolar cell tumors of the lung seen in a dose-related manner in male mice.

Dr. McKnight moved that the Technical Report on 3,3′-dimethylbenzidine dihydrochloride be accepted with the conclusions as written for male and female rats, *clear evidence of carcinogenic activity*. Dr. Davis seconded the motion, which was accepted unanimously with ten votes. Dr. Zeise then moved that mononuclear cell leukemia be added to the conclusion for female rats as "may have been related to chemical administration." Dr. McKnight seconded the motion, which was accepted by nine yes votes to one no vote (Dr. Gold).

INTRODUCTION



3,3′-DIMETHYLBENZIDINE DIHYDROCHLORIDE

CAS No. 612-82-8

$C_{14}H_{16}N_2 \cdot 2HCl$ Molecular Weight 285.2

Synonyms: *o*-Tolidine dihydrochloride; 3,3′-Dimethylbiphenyl-4,4′-diamine dihydrochloride; 3,3′-Dimethylbiphenyl-4,4′-biphenyldiamine dihydrochloride; 4,4′-Diamino-3,3′-dimethylbiphenyl dihydrochloride

USE AND PRODUCTION

3,3′-Dimethylbenzidine dihydrochloride is a yellow crystalline powder that is slightly soluble in water and very soluble in ethanol, ethyl ether, and dilute acids. It is used principally as an intermediate in the production of commercial bisazobiphenyl dyes for coloring textiles, paper, plastic, rubber, and leather. The amine groups of 3,3′-dimethylbenzidine are chemically linked with other aromatic amines in the synthesis of the bisazobiphenyl dyes. The National Institute of Occupational Safety and Health (NIOSH) lists approximately 480 dyes based on 3,3′-dimethylbenzidine, 96 of which were produced in 1981 (NIOSH, 1983). 3,3′-Dimethylbenzidine is also used as a laboratory reagent for the detection of blood and for the colorimetric determination of chlorine in air and water (IARC, 1972).

3,3′-Dimethylbenzidine is manufactured by reducing *o*-nitrotoluene to hydrazotoluene with alkali and then reacting the hydrazotoluene with hydrochloric acid to yield 3,3′-dimethylbenzidine and other by-products (*Kirk-Othmer*, 1978). The production and use of benzidine congeners and benzidine-derived dyes decreased in the United States after reports that benzidine was carcinogenic. According to the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry, the benzidine congeners currently used in the United States as intermediates are imported (personal communication from T. Helmes to D. Morgan, 1989). No recent United States production data for 3,3′-dimethylbenzidine were found; however, available import data show that approximately 34,200 kg of 3,3′-dimethylbenzidine came through principal U.S. customs districts in 1984 (USITC, 1984).

EXPOSURE

Exposure to 3,3′-dimethylbenzidine may occur by inhalation, ingestion, and skin absorption (Meigs *et al.*, 1951, 1954; El-hawari *et al.*, 1979). Occupational exposure to 3,3′-dimethylbenzidine may occur during the manufacture of dyes of which 3,3′-dimethylbenzidine is a chemical intermediate or during handling of the finished 3,3′-dimethylbenzidine-based dyes, where residual amounts of 3,3′-dimethylbenzidine may be present due to incomplete dye synthesis. There is also evidence to suggest that 3,3′-dimethylbenzidine-based dyes are metabolized back to the parent compound *in vivo*, resulting in exposure to 3,3′-dimethylbenzidine. Exposure of workers to 3,3′-dimethylbenzidine may also occur in clinical and analytical chemistry laboratories when 3,3′-dimethylbenzidine is used for the detection of blood or in the quantitation of chlorine in water and glucose by the glucose oxidase method (IARC, 1972; Collier, 1974).

Approximately 1,000 workers are exposed to benzidine, benzidine congeners, and benzidine-derived dyes in dye manufacturing, and approximately 10,000 workers in the various application industries (DETO, 1980). Since many benzidine compounds may exist simultaneously within the same industry, it is difficult to estimate the numbers of exposed workers and extent of exposure to 3,3′-dimethylbenzidine alone. A recent survey estimates there is a potential for exposure to 3,3′-dimethylbenzidine for approximately 10,000 U.S. employees (NIOSH, 1989).

Nonoccupational exposure to 3,3′-dimethylbenzidine-based dyes may occur through contact with materials colored with these dyes or through the use of packaged dyes and paints containing 3,3′-dimethylbenzidine. No estimates of consumer exposure to 3,3′-dimethylbenzidine alone could be found.

METABOLISM

Reductive metabolism of 3,3′-dimethylbenzidine-based dyes produces 3,3′-dimethylbenzidine (Figure 1). Azo reduction can occur either in the liver, via the hepatic 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 (Walker, 1970). Thus, reductive cleavage of benzidine-congener azo dyes is thought to occur primarily by bacterial action in the intestinal tract (Martin and Kenelly, 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′-Dimethylbenzidine-based dyes are metabolized to 3,3′-dimethylbenzidine in dogs and rats (Lynn *et al.*, 1980) and also in humans (Boeniger, 1980). Following exposure to 3,3′-dimethylbenzidine-based dyes, 3,3′-dimethylbenzidine was detected in the urine of dogs and rats at levels greater than the amount that could be accounted for by contamination of the dyes with 3,3′-dimethylbenzidine (Lynn *et al.*, 1980). Dogs metabolized the dyes Direct Blue 25 and Acid Red 114 to 3,3′-dimethylbenzidine and excreted it in urine. Rats metabolized Direct Blue 25 to 3,3′-dimethylbenzidine and N-acetyl-3,3′-dimethylbenzidine, with urine concentrations of 3,3′-dimethylbenzidine comparable to those observed for dogs. However, rats given Acid Red 114 excreted only trace amounts of 3,3′-dimethylbenzidine in urine. Neither dogs nor rats excreted measurable amounts of 3,3′-dimethylbenzidine in the urine after administration of Direct Red 2 or Direct Red 39.

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

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

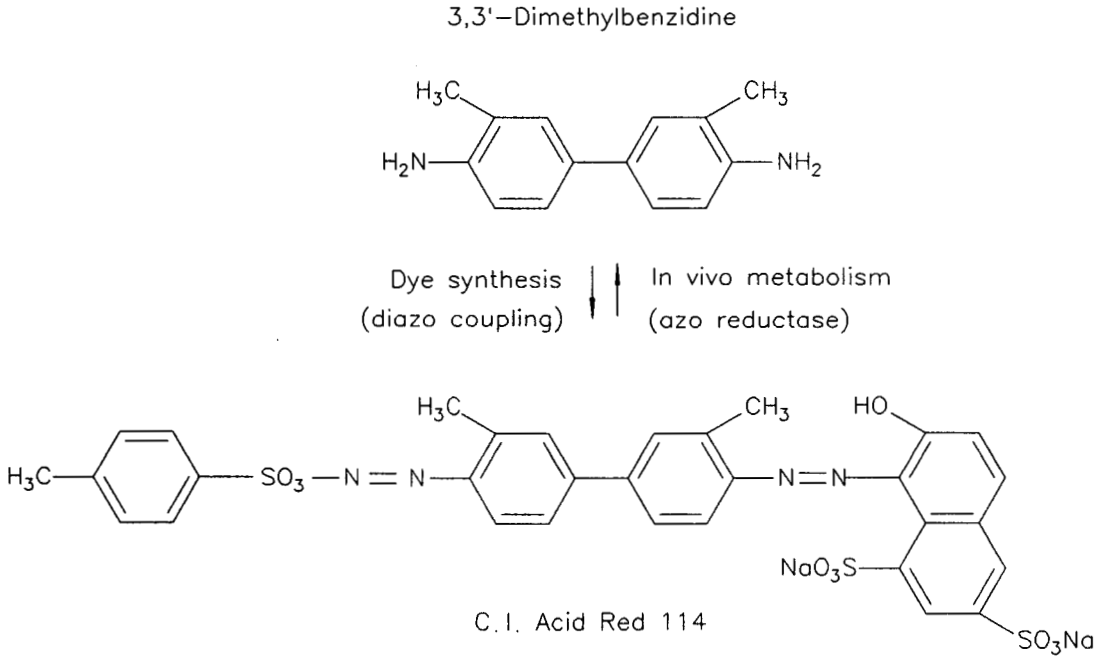


FIGURE 1
Formation of 3,3'-Dimethylbenzidine by Reductive Metabolism of C.I. Acid Red 114

GENETIC TOXICOLOGY

The only available mutagenicity information on the dihydrochloride salt of 3,3'-dimethylbenzidine is included in the NTP test data in this report. Induction of frameshift-type gene mutations occurred in *Salmonella* strain TA98 in the presence of S9 metabolic activation (Zeiger *et al.*, 1988; Table C1). Induction of sister chromatid exchanges (SCE) and chromosome aberrations occurred in cultured Chinese hamster ovary (CHO) cells without S9 metabolic activation (Tables C2 and C3). Induction of sex-linked recessive lethal mutations occurred in germ cells of male *Drosophila* fed or injected with the chemical; however, induction of reciprocal translocations did not occur (Valencia *et al.*, 1985; Tables C4 and C5).

3,3'-Dimethylbenzidine is genotoxic in bacterial and eukaryotic systems. 3,3'-Dimethylbenzidine induced gene mutations in frameshift-sensitive *Salmonella* strains TA98 and TA1538 only in the presence of S9 metabolic activation (Shimizu and Takemura, 1976; Hartman *et al.*, 1978; Martin and Kennelly, 1981; Waalkens *et al.*, 1981; Haworth *et al.*, 1983; Reid *et al.*, 1984a). Two metabolites of 3,3'-dimethylbenzidine, N,N'-diacetyl-3,3'-dimethylbenzidine and N-acetyl-3,3'-dimethylbenzidine, were both positive in *Salmonella* strains TA98, TA100, and TA1538 in the presence of S9 metabolic activation (Tanaka *et al.*, 1982; Kennelly *et al.*, 1984; Reid *et al.*, 1984a). 3,3'-Dimethylbenzidine induced trifluorothymidine resistance in mouse L5178Y lymphoma cells with and without S9 metabolic activation (Mitchell *et al.*, 1988; Myhr and Caspary, 1988). 3,3'-Dimethylbenzidine also gave positive results in *in vitro* mammalian cell assays for the induction of unscheduled DNA synthesis (UDS) (Martin *et al.*, 1978), DNA repair (Kornbrust and Barfknecht, 1984), SCE (Waalkens *et al.*, 1981; Galloway *et al.*, 1987), and chromosomal aberrations (Galloway *et al.*, 1987). The UDS and DNA repair assays were both conducted with S9 metabolic activation. The cytogenetic tests were performed with and without metabolic activation, and positive results were

obtained under both conditions. Another report cites the induction of micronuclei in bone-marrow polychromatic erythrocytes in male Wistar rats given 3,3′-dimethylbenzidine by gavage (Cihak, 1979).

Mutagenicity data for closely related structural analogs of 3,3′-dimethylbenzidine are consistent with the positive results reported above. NTP Technical Report No. 372 (NTP, 1990a) presents a detailed review of the test results for 3,3′-dimethoxybenzidine. This compound tested positive for mutagenic toxicity in *Salmonella* strains TA98, TA100, and TA1535 and induced SCE and chromosomal aberrations in CHO cells, but did not induce sex-linked recessive lethal mutations in adult male *Drosophila.*

Benzidine, the parent compound in this series of substituted biphenyls, induced gene mutations in *Salmonella* strains TA98, TA100, and TA1538 in the presence of S9 metabolic activation (Ames *et al.*, 1973; Shimizu and Takemura, 1976; Anderson and Styles, 1978; Baker and Bonin, 1981; Probst *et al.*, 1981; Haworth *et al.*, 1983; Reid *et al.*, 1984b). Benzidine also induced gene mutations in some strains of *Escherchia coli* in the presence of S9 metabolic activation (Matsushima *et al.*, 1981; Mohn *et al.*, 1981; Venitt and Crofton-Sleigh, 1981). Benzidine and/or its dihydrochloride salt also gave positive results in a variety of *in vitro* eukaryotic genotoxicity assays. It induced mitotic aneuploidy (Parry and Sharp, 1981) and gene conversion (Zimmermann and Scheel, 1981; Sharp and Parry, 1981) in *Saccharomyces*, UDS in mouse primary hepatocyte cultures (Williams, 1978; Probst *et al.*, 1981; Althaus *et al.*, 1982), and gene mutation in mouse L5178Y lymphoma cells (Jotz and Mitchell, 1981; Mitchell *et al.*, 1988; Myhr and Caspary, 1988). Benzidine also induced SCE and chromosomal aberrations in CHO cells (Natarajan and van Kesteren-van Leeuwen, 1981; Galloway *et al.*, 1987) and human lymphoblastoid cells (Tohda *et al.*, 1980). The *in vivo* administration of benzidine induced UDS in rat hepatocytes (Mirsalis *et al.*, 1982) and micronuclei (Salamone *et al.*, 1981; NTP, unpublished), SCE (Parodi *et al.*, 1983; NTP, unpublished), and chromosomal aberrations (NTP, unpublished) in mouse bone marrow cells.

TOXICITY AND CARCINOGENICITY STUDIES

The National Institute of Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) issued a health hazard alert in 1980 stating that persons working with 3,3′-dimethylbenzidine-, benzidine-, and 3,3′-dimethoxybenzidine-based dyes should be aware of the potential health hazards associated with excess exposure (Boeniger, 1980). In a later report issued to alert workers to the hazards of benzidine-congener dyes, NIOSH stated that exposure to 3,3′-dimethylbenzidine-based dyes in the workplace may pose a carcinogenic risk (NIOSH, 1983). These health alerts were based on evidence from animal studies indicating that 3,3′-dimethylbenzidine is carcinogenic and on preliminary evidence indicating metabolic conversion of the 3,3′-dimethylbenzidine-based dyes to the parent compound, 3,3′-dimethylbenzidine. These early carcinogenicity studies of 3,3′-dimethylbenzidine have been criticized for their small numbers of study animals, lack of concurrent controls, use of toxic doses, and use of parenteral routes of chemical administration (Haley, 1975; DETO, 1980).

Spitz *et al.* (1950) treated Sherman rats with weekly subcutaneous doses of 60 mg 3,3′-dimethylbenzidine in olive oil. Treatment-related mortality was high, with 43 of 105 animals dying before day 200 and only 48 animals surviving longer than 300 days. The only significant lesions observed were tumors of the auditory canal, probably Zymbal's gland tumors, in five rats; the first of these tumors was observed on day 354. No auditory canal tumors were among the 56 tumors observed in 578 untreated rats of the same colony. This investigation lacked a concurrent control group.

Pliss (1963) gave rats weekly subcutaneous injections of 20 mg 3,3′-dimethylbenzidine for 13 months. An unspecified number of animals died during the first 2 weeks of the study, and the number of animals surviving the treatment was not indicated. A variety of tumors appeared, primarily between months 14 and 22, with Zymbal's gland tumors occurring most frequently. Lesions also appeared in the liver, mammary gland, gastrointestinal tract, and skin.

Griswold *et al.* (1968) treated 20 female Sprague-Dawley rats with a suspension of 3,3′-dimethylbenzidine in sesame oil. The total dose of 500 mg per rat was divided into 10 equal doses given by stomach tube at 3-day intervals. Animals were observed for 9 months. Mammary gland tumors developed in 3/16 survivors, and, among 132 vehicle control rats, five had a total of three carcinomas, one fibroadenoma, and five hyperplasias of the mammary gland. Neoplasms were not seen at other sites.

No tumors were observed in a lifespan study on groups of 30 male and 30 female hamsters given 3,3′-dimethylbenzidine at 0.1% or 0.3% (the highest tolerated level) in the diet (Saffiotti *et al.*, 1967; Sellakumar *et al.*, 1969).

Pliss and Zabezhinsky (1970) gave 27 male and 26 female rats a 4% suspension of 3,3′-dimethylbenzidine (20 mg per rat) in 0.5 mL sunflower oil by subcutaneous injection once weekly for 13 months. Fifty rats survived to 8 months, after which time the first tumor was observed. Thirty rats developed a total of 41 tumors, including 20 Zymbal's gland carcinomas; neoplasms of the mammary gland, preputial gland, forestomach, skin, lung, liver, thyroid, and uterus were also seen. Tumor production at distant body sites after subcutaneous injection is considered a reliable indication of carcinogenicity (IARC, 1986).

In the same study, two groups of rats were subcutaneously implanted with pellets containing 20 mg 3,3′-dimethylbenzidine and 10 mg glycerol. Pellets implanted in the first group (20 per sex) were not ultraviolet irradiated. The pellets implanted in the second group (24 per sex) were ultraviolet irradiated before implantation to investigate the effects of oxidation on the carcinogenicity of 3,3′-dimethylbenzidine. The differences between the two groups were minimal. From a total of 68 rats alive at the first tumor observation (11-12 months), 48 rats developed a total of 60 tumors. Twenty-seven of these were Zymbal's gland carcinomas, with neoplasms of the mammary gland, skin, liver, and hematopoietic system accounting for the remainder. Although control groups were not monitored during these experiments, a preliminary report of these studies states that rats from the same colony did not develop tumors of the Zymbal's gland (Pliss, 1965).

In more recent studies, the National Center for Toxicological Research (NCTR), as part of the Benzidine Dye Initiative, exposed BALB/c mice (120 per sex per group) to 0, 5, 9, 18, 35, 70, or 140 ppm 3,3′-dimethylbenzidine dihydrochloride in drinking water (Schieferstein *et al.*, 1989, 1990). Groups of mice of each sex were killed after 3, 6, 9, 12, 15, or 24 months of exposure. There were no treatment-related effects on body weights or on water or food consumption. Treatment-related increases in the incidence of fatal lung alveolar cell bronchial adenomas, bronchial carcinomas, and combinations of these were observed in males only. Nonfatal lung tumors did not show a significant dose-related trend. Fatal lung tumors appeared around 78 weeks in mice exposed to 140 ppm 3,3′-dimethylbenzidine; a treatment-related decrease in survival resulting from fatal lung neoplasms was also noted.

TOXICITY/CARCINOGENICITY OF RELATED COMPOUNDS

Benzidine

3,3′-Dimethylbenzidine is a congener of benzidine, 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; Littlefield *et al.*, 1983). Occupational exposure to benzidine for up to 30 years resulted in bladder tumors 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 bladder tumors in dogs (Spitz *et al.*, 1950); hepatocellular, harderian gland, and lymphoreticular tumors in mice (Bonser *et al.*, 1956; Vesselinovitch *et al.*, 1975; Frith and Dooley, 1976); Zymbal's gland, hepatic, and mammary gland carcinomas in rats (Spitz *et al.*, 1950; Griswold *et al.*, 1968); and hepatocellular carcinomas, adenomas, and cholangiomas in hamsters (Saffiotti *et al.*, 1967). Animal survival was poor in many of the carcinogenicity studies of benzidine. Although this was due, in most cases, to the administration of toxic

doses, these studies do assert that benzidine is carcinogenic in laboratory animals.

o-Toluidine

o-Toluidine hydrochloride (2-aminotoluene) is structurally analogous to one-half the 3,3′-dimethylbenzidine molecule. In studies performed by the National Cancer Institute, *o*-toluidine hydrochloride was given to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex in feed at dose levels of 3,000 or 6,000 ppm for rats and 1,000 or 3,000 ppm for mice for 101 to 104 weeks (NCI, 1979a). Twenty untreated animals of each sex and species were used as controls.

Exposure of rats to *o*-toluidine hydrochloride resulted in sarcomas of the spleen and other organs in both males and females. *o*-Toluidine hydrochloride induced mesotheliomas of the abdominal cavity and scrotum in males and transitional-cell carcinomas of the urinary bladder in females. Administration of *o*-toluidine also resulted in increased incidences of fibromas of the subcutaneous tissue in males and fibroadenomas or adenomas of the mammary gland in females. In mice, hemangiosarcomas occurred at various sites in males, and hepatocellular carcinomas or adenomas of the mammary gland occurred in females.

3,3′-Dimethoxybenzidine

The Benzidine Dye Initiative included the evaluation of 3,3′-dimethoxybenzidine dihydrochloride for carcinogenicity (NTP, 1990a). F344/N rats of each sex received 3,3′-dimethoxybenzidine dihydrochloride in drinking water at either 80, 170, or 330 ppm for 21 months. These studies used 50 untreated animals of each sex as controls. After 9 months, neoplastic effects attributed to 3,3′-dimethoxybenzidine dihydrochloride exposure were noted. After exposure for up to 21 months, neoplasms were observed at many sites, including the skin, Zymbal's gland, preputial and clitoral glands, oral cavity, small and large intestine, liver, brain, mesothelium, mammary gland, and uterus and cervix.

Pliss (1963, 1965) treated rats with 30 mg 3,3′-dimethoxybenzidine three times per week via sunflower oil gavage. Because of poor survival, the dose level was reduced to 15 mg after 3 weeks and continued at that level for 13 months. Of the 42 rats starting the study, 18 survived for 14 months. Two survivors exhibited tumors of the Zymbal's gland, and one, an ovarian tumor. None of the 50 control rats developed tumors at the same sites as the exposed rats.

In a lifespan study, Saffiotti *et al.* (1967) treated groups of 30 golden hamsters per sex with 1,000 ppm (0.1% w/w) 3,3′-dimethoxybenzidine in the diet. A small transitional-cell carcinoma of the urinary bladder was found in one animal after 144 weeks of exposure. This tumor is rare in hamsters and was attributed to treatment with 3,3′-dimethoxybenzidine. The same investigators conducted a similar study on hamsters that used a higher dose of 3,3′-dimethoxybenzidine (1.0%) (Sellakumar *et al.*, 1969). Forestomach papillomas occurred in 57% of the treated animals and in only 2% of the controls. No bladder lesions were detected at these dietary concentrations. This publication is an abstract and does not detail the experimental design or survival data.

In a gavage study, Hadidian *et al.* (1968) gave 30 male and 30 female Fischer rats 0.1, 0.3, 1.0, 3.0, 10, or 30 mg 3,3′-dimethoxybenzidine per animal per day, 5 days per week. The vehicle was a proprietary mixture composed of NaCl, carboxymethylcellulose, polysorbate 80, and benzyl alcohol in water. Animals received these dose formulations for 52 weeks, after which they were observed for 6 months and necropsied. Tumors occurred as early as 293 days, but most were detected at necropsy. The variety of tumors reported at necropsy included neoplastic lesions of the urinary bladder (two papillomas), mammary gland (three carcinomas, two fibroadenomas), skin (five carcinomas), intestinal tract (two carcinomas), and Zymbal's gland (eight carcinomas). Tumor incidences were significantly increased over those observed for 360 pooled vehicle and nonvehicle control rats.

o-Anisidine

o-Anisidine (2-methoxyaniline) is structurally analogous to one-half the 3,3′-dimethoxybenzidine molecule. *o*-Anisidine is used in the manufacture of monoazo dyes by diazotization and coupling with other aromatic amines (Noller, 1965). The National Cancer Institute (NCI, 1978a) found that *o*-anisidine was carcinogenic to F344/N rats and B6C3F$_1$ mice. Groups of 55 rats of each sex received *o*-anisidine in feed at 5,000 or 10,000 ppm for 103 weeks; similar

groups of mice received 2,500 or 5,000 ppm. Fifty-five untreated animals of each sex and species were used as controls.

Treatment with *o*-anisidine resulted in transitional-cell carcinomas or papillomas of the bladder in both sexes of each species. Male rats also exhibited transitional-cell carcinomas of the renal pelvis and follicular-cell tumors of the thyroid tissue. Only one urinary system tumor was observed in the control groups of rats or mice, a transitional cell papilloma of the renal pelvis in a male mouse.

STUDY RATIONALE

Benzidine is a known carcinogen (IARC, 1972; 1987) and 3,3′-dimethylbenzidine, a benzidine congener, is possibly carcinogenic for humans (IARC, 1987). Numerous benzidine and ben- zidine congener-based dyes are metabolized to these parent amines *in vivo* (Rinde and Troll, 1975; Lynn *et al.*, 1980). Thus, all benzidine- and benzidine congener-derived dyes are logical candidates for carcinogenicity testing.

The National Toxicology Program's (NTP) Benzidine Dye Initiative is a collaborative effort of NIEHS, NCTR, NIOSH, the U.S. Environmental Protection Agency, the Consumer Product Safety Commission, and OSHA under the aegis of the NTP. The objective of this Initiative is to develop an integrated body of scientific data concerning the metabolism, pharmacokinetics, genetic toxicology, and *in vivo* carcinogenicity of dyes derived from benzidine, 3,3′-dimethylbenzidine, and 3,3′-dimethoxybenzidine (Table 1). 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.

TABLE 1
Summary of the National Toxicology Program Benzidine Congener Initiative

Class/Chemical	Tests[a]
3,3′-Dimethylbenzidine (*o*-tolidine)	
o-Tolidine	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.

The agencies collaborating in the Benzidine Dye Initiative selected 3,3′-dimethylbenzidine for study to allow comparison of its toxic and carcinogenic effects with those of related chemicals studied simultaneously with comparable doses and study designs. 3,3′-Dimethylbenzidine was also studied to strengthen the evidence for its carcinogenicity. Although results of earlier studies suggest that 3,3′-dimethylbenzidine is carcinogenic (Griswold *et al.*, 1968; Hadidian *et al.*, 1968; Pliss and Zabezhinsky, 1970), these studies were criticized for their use of small numbers of study animals, lack of concurrent controls, use of toxic doses, and use of parenteral routes of chemical administration (Haley, 1975; DETO,1980).

3,3′-Dimethylbenzidine dihydrochloride is one of five benzidine congeners or benzidine congener-derived dyes selected for evaluation in the 2-year carcinogenicity studies as part of the Benzidine Dye Initiative. The other chemicals studied are 3,3′-dimethoxybenzidine dihydrochloride (a related benzidine congener), C.I. Direct Blue 15 and C.I. Direct Blue 218 (representative 3,3′-dimethoxybenzidine-based dyes), and C.I. Acid Red 114 (a representative dimethylbenzidine-based dye). The oral route of administration was selected for these studies to maximize the chances of detecting systemic effects associated with chemical administration. These studies used the same design. The instability of 3,3′-dimethylbenzidine dihydrochloride and 3,3′-dimethoxybenzidine dihydrochloride in feed required administration of these chemicals in drinking water. Because long-term studies of 3,3′-dimethylbenzidine dihydrochloride and 3,3′-dimethoxybenzidine dihydrochloride were being conducted on mice at NCTR, the NTP studies of these chemicals used only rats.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 3,3′-DIMETHYLBENZIDINE DIHYDROCHLORIDE

3,3′-Dimethylbenzidine dihydrochloride was obtained from the Taylor Chemical Company in two lots. Lot number T122380 was used in both the 14-day and 13-week studies, and lot number IP22 was used in the 14-month studies. Purity and identity analyses were conducted at the Midwest Research Institute, Kansas City, MO (Appendix F). The study chemical in both lots was identified as 3,3′-dimethylbenzidine dihydrochloride by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of both lots was determined to be 99% by elemental analysis, Karl Fischer water analysis, titrations (non-aqueous amine and neutralization titrations), thin-layer chromatography, and high-performance liquid chromatography (HPLC). Comparison of the two lots by HPLC showed no significant purity differences. The test laboratory confirmed the chemical identity by infrared spectroscopy, and the stability, by HPLC and non-aqueous amine titration. No degradation of the study material was detected by these analytical methods.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Initially, attempts were made to formulate 3,3′-dimethylbenzidine dihydrochloride in feed. The 2-week stability of NIH-07 Rat and Mouse Ration formulated with 675 ppm 3,3′-dimethylbenzidine dihydrochloride was determined at storage temperatures ranging from -20° C to room temperature. Results showed that feed formulations were unstable when stored at or above 5° C. Formulated diets stored open to air and light and under simulated animal room conditions lost 18% or 21% of the chemical after 3 or 7 days, respectively. The same formulations stored in the dark in sealed containers at room temperature, 5° C, or -20° C lost 23%, 15%, or 5% of the chemical following storage for 2 weeks.

Drinking water was then investigated as a vehicle for chemical administration. Tests showed that solutions of 675 ppm 3,3′-dimethylbenzidine dihydrochloride in water remained stable for at least 14 days when stored at either room temperature or 5° C. Solutions were also stable for up to 48 hours under simulated dosing conditions, including exposure to normal room light.

Tap water was used for the preparation of dose formulations during the 14-day studies, and distilled water was used during the 13-week and 14-month studies. Dose formulations were prepared twice weekly and made available to the study animals within 7 days of mixing. The preparation and storage procedures for dosed drinking water in the studies of 3,3′-dimethylbenzidine dihydrochloride 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 14-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 80% of the formulations were prepared within specifications (Table F3). Results of periodic referee analyses by the analytical chemistry laboratory agreed with the results of the study laboratory (Table F4).

14-DAY STUDIES

Male and female F344/N rats were obtained from Frederick Cancer Research Facility (Frederick, MD) and observed for 13 days before the studies began. The rats were 48 days old when placed on study. Groups of five rats of each sex received 0, 600, 1,250, 2,500, 5,000, or 7,500 ppm 3,3′-dimethylbenzidine dihydrochloride 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. Clinical observation of the animals was conducted 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. Complete necropsies were performed on all animals. The following organs were weighed: brain, heart, kidney (right), liver, lung, testicle (right), and thymus. Complete histopathologic examinations were performed on all control animals, males receiving 5,000 and females receiving 7,500 ppm. Selected tissues were examined for the other dose groups. Further details are presented in Table 2.

13-WEEK STUDIES

The 13-week studies were conducted to evaluate cumulative toxic effects of repeated exposure to 3,3′-dimethylbenzidine dihydrochloride and to determine the chemical concentrations to be used in the 2-year studies.

Fischer 344/N rats were obtained from Frederick Cancer Research Facility, observed for 16 days, distributed to weight classes, and assigned to dose groups. The rats had a median age of 55 days when placed on study. Groups of ten rats of each sex received 0, 300, 500, 1,000, 2,000, or 4,000 ppm 3,3′-dimethylbenzidine dihydrochloride 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 study termination. Erythrocyte counts, leukocyte counts, differential leukocyte counts, hemoglobin concentrations, and hematocrit values were determined on samples drawn from the retro-orbital sinus. Clinical chemistry values for blood urea nitrogen (BUN), serum creatinine, lactic dehydrogenase (LDH), sorbitol dehydrogenase (SDH), alanine aminotransferase (ALT), triiodothyronine (T_3), thyroxine (T_4), and thyroid stimulating hormone (TSH) were determined from blood samples collected from the abdominal aorta. T_3 and T_4 were analyzed with the Tri-Tab RIA Diagnostic Kit and the Tetra-Tab Diagnostic Kit (Nuclear Medical Laboratories). TSH analysis was performed by the method of Ridgway *et al.* (1973). Further details are presented in Table 2.

Survivors were killed at the end of the 13-week studies. Necropsies were performed on all study animals. The brain, heart, liver, lung, kidney (right), testis (right), and thymus of survivors were weighed at necropsy. Complete histopathological examinations were performed on all animals in the control and high-dose groups, all animals in the highest dose groups with a survival rate of 60% or less (2,000 ppm dose group), and on all animals that died or were killed moribund. Selected organs were submitted for histopathology for the remaining animals. Tissues examined for each group are listed in Table 2.

9-MONTH AND 14-MONTH STUDIES

Study Design

The 14-month studies were originally designed for 24 months with an animal allocation recommended by Portier and Hoel (1984). At 9 months, ten rats of each sex in the control and 150 ppm dose groups were killed, and at 14 months ten rats of each sex at each dose level were to be killed; animals were predesignated for the 9- and 14-month sacrifices prior to study start. Because of the large number of early deaths in the exposed groups, the study was terminated at 14 months, and the 14-month interim sacrifice animals were added to the core groups, resulting in 60 rats in the control groups, 45 in the 30 ppm groups, 75 in the 70 ppm groups, and 60 in the 150 ppm groups.

Source and Specification of Animals

Male and female F344/N rats were obtained from Frederick Cancer Research Facility (Frederick, MD) for use in the 2-year studies. Breeding stock for the foundation colony at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents and were transferred from isolators to barrier-maintained rooms. The animals were 4 weeks old at receipt. Following a 14-day quarantine, five animals of each sex were randomly selected and sacrificed for parasite evaluation and gross observation of disease. Serology samples were collected for viral screens. Study animals were 6 weeks 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 5 per cage. Feed (Appendix H) and water were available *ad libitum*. Cages were rotated every 2 weeks during the studies. Further details of animal maintenance are given in Table 2.

Clinical Observations and Pathology

All animals were observed twice daily. Animals were weighed at study initiation, weekly for 14 weeks, at week 17, and every 4 weeks thereafter. Clinical findings were noted and recorded at the time of weighing. Feed consumption was measured weekly, and water consumption, twice weekly.

At 9 months, ten rats of each sex from the control and high-dose (150 ppm) groups were killed. Blood and urine samples were collected prior to sacrifice. 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 BUN, creatinine, glucose, ALT, LDH, SDH, T_3, T_4, TSH, and serum osmolality were determined from blood samples collected from the abdominal aorta. T_3, T_4, and TSH were analyzed with the same methods used in the 13-week studies. 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 were weighed at necropsy. Further details are presented in Table 2.

Animals found moribund, designated for the 9-month studies, or surviving to the end of the 14-month studies were killed. Necropsy was performed on all animals. 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 groups examined are listed in Table 2. In some cases, a particular organ or tissue may have been autolyzed or lost; thus, the numbers of organs and tissues examined microscopically vary and are not necessarily equal to the number of animals placed on study.

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

A quality assessment pathologist reviewed selected tissues microscopically for accuracy and consistency of lesion diagnosis. All neoplastic and nonneoplastic lesions were reviewed in the following tissues from all male and female rats: clitoral or preputial gland, liver, lung, kidney, and Zymbal's gland. In addition, all neoplastic diagnoses in tissues other than those already mentioned were reviewed in all animals, and all diagnoses (neoplastic and nonneoplastic) were reviewed from a random 10% of the animals from each control and high-dose group.

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

Statistical Methods

Survival Analyses

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

Calculation of Incidence

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

Analysis of Tumor Incidence

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

For incidental tumors (tumors discovered as a result of death from an unrelated cause), one method of analysis used in this study was logistic regression. 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). However, markedly reduced survival in dosed animals (due largely to the increased incidence of lethal tumors) reduced the power of logistic regression to detect carcinogenic effects in some instances. Therefore, although the results of logistic regression analysis are given in Appendixes A and B for informational purposes, primary emphasis was given to the Cochran-Armitage and Fisher exact tests based upon the *effective number* of animals. The effective number is the number of animals surviving until the appearance of the first tumor. These survival-adjusted procedures are recommended by Gart *et al.* (1979).

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

Historical Control Data

Although the concurrent control group is the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Although the current studies were terminated at 14 months, tumor incidences from the NTP historical control data base for 2-year studies (Haseman *et al.*, 1984, 1985) are included for tumors appearing to show compound-related effects.

Analysis of Continuous Variables

Organ-weight-to-body-weight ratios and hematology and serum chemistry data from the 14-day and 13-week studies were analyzed using the nonparametric comparison procedures 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 Dunn's or Shirley's test was more appropriate for pairwise comparisons. For the 9-month studies (in which a single dose group was compared with the controls), Wilcoxon's rank sum test (Hollander and Wolfe, 1973) was used to evaluate organ weight, hematology, serum chemistry, and urinalysis data.

Quality Assurance Methods

The 13-week and 14-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 draft of the 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 and were resolved or were otherwise addressed during the preparation of this Technical Report.

TABLE 2
Experimental Design and Materials and Methods in the Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

14-Day Studies	13-Week Studies	14-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 Facility (Frederick, MD)	Frederick Cancer Research Facility (Frederick, MD)	Frederick Cancer Research Facility (Frederick, MD)
Time Held Before Study 13 days	16 days	14 days
Age When Placed on Study 48 days	Median age 55 days	42 days
Date of First Dose 22 March 1982	30 July 1982	30 June 1983
Date of Last Dose 5 April 1982	Males: 2 November 1982 Females: 3 November 1982	21-29 August 1984 (dosed until necropsy)
Duration of Dosing 14 consecutive days	13 weeks (7 days/week)	60-61 weeks (7 days/week)
Age at Necropsy 9 weeks	21-22 weeks	66-67 weeks
Necropsy Dates 5 April 1982	Males: 3 November 1982 Females: 4 November 1982	21-29 August 1984
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 punch and ear tag

TABLE 2
Experimental Design and Materials and Methods in the Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

14-Day Studies	13-Week Studies	14-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	Distilled water (Polar Water Co., Beltsville, MD) in glass water bottles with stainless steel sippers (Hazleton Systems, Inc., Aberdeen, MD); available ad libitum	Same as 13-week studies
Cages		
Polycarbonate (Hazleton Systems, Inc., Aberdeen, MD)	Same as 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 polyester nonwoven fiber filters (DuPont Company, Applied Technologies Division, Wilmington, DE)	Same as 14-day studies	Same as 14-day studies
Animal Room Environment		
Temperature: 72°-77° F Humidity: 41%-69% Fluorescent light: 12 hours/day	Temperature: 69°-74° F Humidity: 24%-74% Fluorescent light: 12 hours/day Room air changes: 16.7/hour	Temperature: 65°-92° F Humidity: 25%-80% Fluorescent light: 12 hours/day Room air changes: 10.4/hour
Doses		
0, 600, 1,250, 2,500, 5,000, or 7,500 ppm 3,3′-dimethylbenzidine dihydrochloride in drinking water	0, 300, 500, 1,000, 2,000, or 4,000 ppm 3,3′-dimethylbenzidine dihydrochloride in drinking water	0, 30, 70, or 150 ppm 3,3′-dimethylbenzidine dihydrochloride in drinking water
Type and Frequency of Observation		
Observed twice/day; body weight initially and once/week; feed consumption once/week; water consumption twice/week	Same as 14-day studies	Observed twice/day; body weights initially, once/week for 14 weeks, at week 17, once/month thereafter; feed consumption measured 1 week/month; water consumption measured 1 week/month in 3-day and 4-day segments; clinical observations at body weight determinations

TABLE 2
Experimental Design and Materials and Methods in the Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

14-Day Studies	13-Week Studies	14-Month Studies
Necropsy, Histopathology, and Clinical Pathology Studies		
Necropsy Necropsy performed on all animals. Organ weights obtained at necropsy (brain, heart, liver, lung, right kidney, right testis, and thymus).	**Necropsy** Necropsy performed on all animals. Organ weights measured were the same as in the 14-day studies.	**Necropsy** Necropsy performed on all animals. Organ weights measured at 9-month interim evaluation (brain, kidney, liver).
Histopathology Complete histopathology on male and female controls, 7,500 ppm females, and 5,000 ppm males, including the following organs: adrenal, blood smear, bone (sternebrae, femur, or vertebrae, including marrow), bone marrow (sternum), 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 cavity and turbinates, ovaries, pancreas, parathyroid, pharynx (if grossly abnormal), pituitary, preputial gland, prostate, salivary gland, skin, small intestines (duodenum, ileum, jejunum), spinal cord (if neurological signs present), spleen, stomach, testes, thymus, thyroid, trachea, urinary bladder, uterus, Zymbal's gland, and gross lesions. The following target organs were examined from 5,000 ppm females and 2,500 ppm males and females: adrenal, bone marrow (sternum), epididymis, kidneys, liver, lymph nodes (mandibular, mesenteric), pancreas, spleen, testes, and thymus. The following target organs were examined from 1,250 ppm males: adrenal, bone marrow (sternum), liver, lymph nodes (mandibular, mesenteric), kidneys, spleen, testes, and thymus; from 1,250 ppm females: adrenal, bone marrow (sternum), kidneys, pancreas, and thymus; from 600 ppm females: bone marrow (sternum).	**Histopathology** Complete histopathology on male and female controls, all deaths and moribund kills (all 4,000 ppm males and females and 4 males and 3 females from 2,000 group), all males and females from the 2,000 ppm group surviving to termination. Tissues examined were the same as in the 14-day studies complete screen. The following organs were examined from 1,000 ppm males: adrenal, bone marrow (sternum), kidney, liver, lymph nodes (mandibular, mesenteric), pancreas, spleen, testes, and thymus; from 500 ppm males: kidney, liver, and pancreas; from 300 ppm males: liver and pancreas; from 100 ppm females: bone marrow (sternum), kidney, liver, lymph nodes (mandibular, mesenteric), pancreas, spleen, and thymus; from 500 and 300 ppm females: kidney, liver, and pancreas.	**Histopathology** Complete histopathology on all animals that died, were moribund kills, or were killed at 9 months or termination. Tissues examined same as 14-day studies complete screen with the addition of seminal vesicles.
Clinical Pathology None required	**Clinical Pathology** Clinical pathology studies conducted at 13 weeks. ***Hematology:*** hematocrit, hemoglobin, erythrocytes, leukocytes, segmented neutrophils, lymphocytes, monocytes, eosinophils, and erythrocyte and lymphocyte morphology ***Clinical chemistry:*** blood urea nitrogen, creatinine, triiodothryronine, thyroxine, thyroid-stimulating hormone, lactate dehydrogenase, sorbitol dehydrogenase, alanine aminotransferase	**Clinical Pathology** Clinical pathology studies conducted at 9 months. ***Hematology:*** hematocrit, hemoglobin, erythrocytes, leukocytes, segmented neutrophils, lymphocytes, monocytes, eosinophils, and erythrocyte and lymphocyte morphology ***Clinical chemistry:*** blood urea nitrogen, creatinine, glucose, serum osmolality, triiodothryronine, thyroxine, thyroid-stimulating hormone, lactate dehydrogenase, sorbitol dehydrogenase, alanine aminotransferase ***Urinalysis:*** Protein, glucose, creatinine, pH, specific gravity, urine osmolality, volume, creatinine excretion rate (16 hr), serum/urine osmolality, and microscopic exam of sediment

RESULTS

14-DAY STUDIES

All five males and one female receiving 7,500 ppm 3,3′-dimethylbenzidine dihydrochloride and 1/5 males receiving 5,000 ppm died (Table 3). The final mean body weights of rats receiving 2,500 ppm or more were lower than the initial weights. Depressions in final mean body weight relative to controls ranged from 11% to 60% in male rats receiving at least 1,250 ppm and from 6% to 61% in treated females. Water consumption declined with increasing dose and at 7,500 ppm was less than one-sixth that by controls. Clinical findings included urine stains, skin cold to the touch, rough hair coat, ataxia, and reddish discharge at the eyes and nares in the 7,500 ppm group and thinness and/or kyphosis in other groups.

The absence of body fat was the most notable necropsy observation in animals receiving 5,000 and 7,500 ppm. Gross necropsy findings included small thymus glands in 2,500 and 5,000 ppm males and females and small seminal vesicles in 7,500 ppm males. Significant depressions in the absolute weights and increases in the relative weights of several organs (Tables E1 and E2) reflected the marked decreases in necropsy body weight for animals receiving 2,500, 5,000, and 7,500 ppm. Hepatocyte necrosis and brown pigmentation of the cells lining the hepatic sinusoids were present in males receiving as little as 2,500 ppm and females receiving 5,000 and 7,500 ppm. An increase in the severity of nephropathy and bone marrow hypocellu-

TABLE 3
Survival, Mean Body Weights, and Water Consumption of Rats in the 14-Day Studies of 3,3′-Dimethylbenzidine Dihydrochloride

Concentration (ppm)	Survival[a]	Mean Body Weights (g)			Final Weight Relative to Controls (%)	Water Consumption[d]	
		Initial[b]	Final	Change[c]		Week 1	Week 2
Male							
0	5/5	143 ± 2.7	218 ± 4.8	+74 ± 2.9		24	24
600	5/5	147 ± 2.3	218 ± 3.2	+70 ± 2.0	100	15	18
1,250	5/5	148 ± 2.5	195 ± 2.9	+47 ± 2.4	89	16	17
2,500	5/5	147 ± 1.5	105 ± 8.3	-41 ± 7.1	48	7	8
5,000	4/5[e]	147 ± 1.2	88 ± 3.8	-60 ± 5.0	40	4	4
7,500	0/5[e]	146 ± 1.9	–[f]	–	–	3	3
Female							
0	5/5	117 ± 1.3	153 ± 2.6	+36 ± 1.6		19	17
600	5/5	116 ± 2.3	143 ± 2.1	+27 ± 1.9	94	14	13
1,250	5/5	115 ± 1.0	132 ± 0.7	+17 ± 0.8	86	10	10
2,500	5/5	115 ± 1.0	112 ± 1.2	-3 ± 0.6	73	8	9
5,000	5/5	114 ± 1.4	60 ± 1.0	-54 ± 1.2	39	4	3
7,500	4/5[e]	114 ± 0.5	63 ± 1.4	-52 ± 1.2	41	3	3

[a] Number surviving/number initially in group
[b] Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
[c] Mean body weight change of the survivors ± standard error of the mean
[d] Milliliters per animal per day, based on average consumption data obtained during the 2-week interval
[e] All mortality in these groups occurred by day 13.
[f] No data are reported due to 100% mortality in this group.

larity (atrophy) was associated with exposure to as little as 2,500 ppm 3,3′-dimethylbenzidine dihydrochloride. Treated animals showed moderate to severe lymphocytic atrophy of the thymus and varying degrees of lymphocytic atrophy of the spleen and mandibular and mesenteric lymph nodes. Treated animals also showed necrosis and vacuolation of adrenal cortical cells, focal acinar cell hypertrophy of the pancreas, and, in males, increased numbers of immature sperm forms in the testis and epididymis.

13-WEEK STUDIES

All animals receiving 4,000 ppm 3,3′-dimethylbenzidine dihydrochloride died by week 4 of the study; 4/10 males and 3/10 females receiving 2,000 ppm also died prior to terminal sacrifice (Table 4). The final mean body weight of 2,000 ppm females was lower than the initial weight. Depressions in the final mean body weights of treated rats relative to controls ranged from 12% to 48% in males and from 9% to 42% in females; these depressions were particularly evident in the 2,000 ppm group. By week 7, water consumption by rats in the 2,000 ppm group was about 45% of that by controls. Clinical findings noted during the studies included crusty red exudate on the noses of rats receiving 300 ppm or more, thinness, stains on the fur, and urine stains. These findings appeared as early as week 1 or 2 in animals receiving 4,000 ppm or 2,000 ppm, respectively.

TABLE 4
Survival, Mean Body Weights, and Water Consumption of Rats in the 13-Week Studies of 3,3′-Dimethylbenzidine Dihydrochloride

Concentration (ppm)	Survival[a]	Mean Body Weights (g)			Final Weight Relative to Vehicle Controls (%)	Water Consumption[d]	
		Initial[b]	Final	Change[c]		Week 7	Week 13
Male							
0	10/10	170 ± 2.7	351 ± 7.8	+181 ± 6.5		23	21
300	10/10	168 ± 2.6	307 ± 5.2	+139 ± 4.3	88	19	16
500	10/10	169 ± 2.5	312 ± 4.0	+143 ± 3.6	89	14	14
1,000	10/10	170 ± 2.5	303 ± 3.8	+133 ± 3.4	86	14	14
2,000	6/10[e]	164 ± 4.2	182 ± 16.7	+16 ± 14.1	52	10	10
4,000	0/10[f]	172 ± 4.1	—[g]	—[g]	—[g]	—[k]	—[g]
Female							
0	10/10	120 ± 3.9	198 ± 2.1	+78 ± 4.2		20	24
300	10/10	123 ± 3.6	181 ± 2.2	+58 ± 3.8	91	12	13
500	10/10	126 ± 2.8	180 ± 2.8	+54 ± 2.8	91	10	12
1,000	10/10	118 ± 2.1	166 ± 2.4[j]	+47 ± 2.7	84	8	11
2,000	7/10[h]	122 ± 2.8	115 ± 7.3	-7 ± 9.2	58	9	7
4,000	0/10[i]	123 ± 2.6	—[g]	—[g]	—[g]	—[l]	—[g]

[a] Number surviving/number initially in group
[b] Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
[c] Mean body weight change of the survivors ± standard error of the mean
[d] Milliliters per animal per day, based on average consumption data obtained during the 13-week interval
[e] Week of death: 6, 7, 11, 13; one animal died before completion of terminal sacrifice
[f] Week of death: 2, 2, 3, 3, 3, 3, 4, 4, 4, 4
[g] No data are reported due to 100% mortality in this group.
[h] Week of death: 13; two animals died before completion of terminal sacrifice
[i] Week of death: 3, 3, 3, 3, 3, 3, 3, 4, 4, 4
[j] Mean based on 9 animals; one female inadvertently not weighed at week 13
[k] Water consumption was 5 mL/animal per day for weeks 1 and 2 and 15 mL/animal per day for week 3.
[l] Water consumption was 4 mL/animal per day for week 1, 5 mL/animal per day for week 2, and 13 mL/animal per day for week 3.

Mean necropsy body weights were significantly decreased in rats at all dose levels, making changes in absolute and relative organ weights more difficult to evaluate. The only consistent effect was a reduction in thymus weight in females at all dose levels (Tables E3 and E4). Significant decreases in hematocrit and erythrocyte count were observed in males receiving 1,000 and 2,000 ppm and in females receiving 500 ppm or more (Table D1). Males and females at all dose levels showed elevated sorbitol dehydrogenase (SDH) levels; alanine aminotransferase was increased only in the 1,000 ppm male and 2,000 ppm female groups. Males receiving 300 or 1,000 ppm showed significant elevations in lactate dehydrogenase. Slight increases in mean blood urea nitrogen (BUN) levels were observed in male rats receiving 2,000 ppm. Decreases in mean creatinine observed in females receiving 300 and 500 ppm were not considered compound related. Triiodothyronine (T_3) values were significantly decreased in exposed females, and thyroxin (T_4) values were significantly decreased in exposed males and females. No significant changes in thyroid stimulating hormone (TSH) levels were observed in exposed rats.

A paucity of body fat and the presence of reddened areas in the glandular mucosa of the stomach were the most notable necropsy findings. Significant histomorphologic alterations were observed in the liver, kidney, bone marrow, lymphoid organs (spleen, mandibular and mesenteric lymph nodes, and thymus), pancreas, and testis of treated rats (Table 5). Hepatic damage, including minimal to moderate hepatocyte necrosis (scattered individual hepatocytes) and brown pigment within the sinusoidal lining cells, was observed in males and females receiving 2,000 and 4,000 ppm and, to a lesser extent, in females receiving 300 ppm and more and in one male receiving 300 ppm. Males and females receiving 1,000 ppm and more and females receiving 500 ppm showed an increased severity of nephropathy over controls; some females receiving 500, 1,000, or 2,000 ppm exhibited karyomegaly of renal tubule epithelial cells. Bone marrow hypocellularity (atrophy) was observed at doses of 2,000 and 4,000 ppm and was consistent with clinical pathology findings. Lymphocytic atrophy of the thymus, spleen, and mandibular and mesenteric lymph nodes was observed in the 2,000 and 4,000 ppm dose groups; females receiving 1,000 ppm also showed lymphocytic atrophy of the thymus. Animals receiving 2,000 and 4,000 ppm showed pancreatic acinar degeneration, and males receiving 1,000, 2,000, or 4,000 ppm showed immature sperm forms in the testis and epididymis; these changes were considered possibly secondary to the general physical debilitation of the study animals.

Dose Selection Rationale

Because of reduced survival in the groups receiving 2,000 or 4,000 ppm, dose-related depressions in weight gain and water consumption, and evidence of compound-related hepatocellular and renal damage and bone marrow hypocellularity (atrophy) in the 13-week studies, drinking water concentrations of 3,3′-dimethylbenzidine dihydrochloride selected for rats in the 9-month and 14-month studies were 30, 70, and 150 ppm.

9-Month Studies

Mean necropsy body weights of animals receiving 150 ppm 3,3′-dimethylbenzidine dihydrochloride were decreased significantly relative to controls. Mean absolute and relative liver and kidney weights and relative brain weights for animals receiving 150 ppm were significantly greater than those for controls (Tables E5 and E6). Moderate decreases in hematocrit, hemoglobin, and erythrocyte counts were observed in high-dose animals (Table D2). Increases in creatinine in males, blood glucose in females, and SDH in both sexes (more pronounced in females) were observed in treated animals. Although T_3 assay results for males and females conflicted, decreases in T_4 and increases in TSH were recorded for high-dose males and females; these alterations were not accompanied by histologic changes in the thyroid gland. Increased urine osmolality, urine/serum osmolality, urine creatinine, urine specific gravity, and protein concentration were observed in high-dose animals. The increase in protein concentration was likely only a reflection of low urine volume.

After exposure to 3,3′-dimethylbenzidine dihydrochloride at 150 ppm for only 9 months, a variety of treatment-related lesions were found, including neoplastic nodules (hepatocellular adenomas) and hepatocellular carcinoma of the liver; proliferative epithelial lesions of the Zymbal's gland, preputial and clitoral glands, and oral cavity, including squamous papilloma and carcinoma, adenomas, carcinomas, and focal hyperplasia; epithelial neo-

TABLE 5
Incidences of Treatment-Related Lesions in Rats in the 13-Week Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

Lesion	0 ppm	300 ppm	500 ppm	1,000 ppm	2,000 ppm	4,000 ppm
Male						
Liver						
Individual hepatocyte necrosis	0/10	0/10	0/10	0/10	7/10**	3/10
Pigment	0/10	1/10	0/10	0/10	10/10**	9/10**
Kidney						
Nephropathy	10/10 (1.1)[a]	–[b]	10/10 (1.0)	10/10 (1.6)	10/10 (2.6)	10/10 (2.6)
Thymus						
Lymphocytic atrophy	0/10	–	–	0/10	5/6**	9/9**
Spleen						
Lymphocytic atrophy	0/10	–	–	0/10	5/10*	10/10**
Mandibular Lymph Node						
Lymphocytic atrophy	0/10	–	–	0/10	7/10**	10/10**
Mesenteric Lymph Node						
Lymphocytic atrophy	0/10	–	–	0/10	1/10	2/9
Bone Marrow						
Hypocellularity	0/10	–	–	0/10	8/10**	10/10**
Pancreas						
Degeneration[c]	0/10	–	–	0/10	4/10*	10/10**
Testes						
Immature sperm	0/10	–	–	1/10	3/10	7/10**
Female						
Liver						
Individual hepatocyte necrosis	0/10	1/10	6/10**	4/10*	7/10**	7/9**
Pigment	0/10	10/10**	10/10**	10/10**	9/10**	8/9**
Kidney						
Nephropathy	2/10 (1.0)	5/10 (1.0)	10/10** (1.0)	10/10** (1.0)	10/10** (2.2)	7/9** (2.1)
Karyomegaly[d]	0/10	0/10	0/10	7/10**	9/10**	0/9
Thymus						
Atrophy	0/10	–	–	2/10	7/8**	5/5**
Spleen						
Atrophy	0/10	–	–	0/10	4/10*	9/9**
Mandibular Lymph Node						
Atrophy	1/10	–	–	0/10	5/10	7/7**
Mesenteric Lymph Node						
Atrophy	0/10	–	–	0/10	4/10*	6/7**
Bone Marrow						
Hypocellularity	0/10	–	–	0/10	10/10**	9/9**
Pancreas						
Degeneration	0/10	–	–	–	2/10	8/9**

* Significantly different ($P \leq 0.05$) from the control group by Fisher exact test
** $P \leq 0.01$
[a] Values in parentheses are average severity grades for affected animals; 1=minimal, 2=slight, 3=moderate.
[b] Organ not examined in animals at this dose level.
[c] Terminology preferred by Pathology Working Group for the lesion diagnosed as acinar hypertrophy by the laboratory pathologist.
[d] Terminology preferred by Pathology Working Group for the lesion diagnosed as megalocytosis by the laboratory pathologist.

plasms of the skin, including squamous cell papilloma, sebaceous gland adenoma, and basal cell carcinoma; mucinous adenocarcinoma of the small intestine; adenomatous polyp of the colon; hyperplasia of the alveolar epithelium; invasive alveolar/bronchiolar carcinoma; and lymphoid atrophy of the spleen (Table 6). Nonneoplastic changes, including hepatocellular hypertrophy, basophilic foci, fatty change, and cystic degeneration, occurred in the liver of treated animals. The severity of nephropathy was increased in treated males and females, and the incidence of nephropathy was increased in treated females as compared to controls. Moderate chronic nephropathy was observed in all treated animals and was consistent with observed changes in kidney weights and BUN and creatinine levels.

TABLE 6
Incidences of Treatment-Related Lesions in Rats in the 9-Month Drinking Water Studies of 3,3′-Dimethylbendizine Dihydrochloride

	Male		Female	
	0 ppm	150 ppm	0 ppm	150 ppm
Number of animals examined	10	10	10	10
Liver				
Hepatocellular carcinoma	0	2	0	0
Neoplastic nodule[a]	0	5*	0	1
Hepatocyte hypertrophy	0	10**	0	10**
Basophilic focus	0	10**	0	0
Fatty change[b]	1	10**	0	10**
Cystic degeneration	0	7**	0	0
Lung				
Alveolar/bronchiolar carcinoma	0	1	0	0
Alveolar/bronchiolar adenoma	0	0	0	1
Alveolar epithelium hyperplasia	0	7**	0	1
Skin				
Basal cell carcinoma	0	1	0	0
Sebaceous gland adenoma	0	1	0	0
Squamous papilloma	0	0	0	1
Oral Cavity (Palate)				
Squamous cell carcinoma	0	0	0	1
Preputial/Clitoral Gland				
Adenoma	0	1	0	2
Carcinoma	0	2	0	3
Small Intestine				
Mucinous adenocarcinoma	0	2	0	0
Large Intestine				
Adenomatous polyp	0	3	0	0
Zymbal's Gland				
Carcinoma	0	2	0	3
Adenoma	0	1	0	2
Squamous papilloma	0	3	0	1
Squamous hyperplasia	0	3	0	1
Focal hyperplasia	0	1	0	0
Kidney				
Nephropathy[c]	10 (1.0)	10 (3.4)	3 (1.0)	10 (3.0)
Spleen				
Lymphoid atrophy[d]	0	10**	0	7**

* Significantly different (P≤0.05) from the control group by Fisher exact test
** P≤0.01
a Term previously used for lesions currently classified as hepatocellular adenoma.
b Diagnosed as cytoplasmic vacuolization by the study pathologist.
c Values in parentheses are average severity grades; 1=minimal, 2=mild, 3=moderate, and 4=marked.
d Diagnosed as lymphoid depletion by the study pathologist.

14-MONTH STUDIES

Body Weights, Water Consumption, and Clinical Findings

The mean body weight of males receiving 150 ppm was approximately 85% of that of controls by week 29 and about 70% of the mean control value by study termination. By week 33, mean body weight for females receiving 150 ppm was approximately 85% of the control mean body weight, and by week 45 the same was true for females receiving 70 ppm (Tables 7 and 8 and Figure 2). The average daily water consumption per rat in the low-, mid-, and high-dose groups was 95%, 91%, and 105% that by controls for males and 101%, 96%, and 79% for females (Tables G1 and G2). The average amount of 3,3′-dimethylbenzidine dihydrochloride consumed per day was approximately 1.8, 4.0, or 11.2 mg/kg for low-, mid-, or high-dose males and 3.0, 6.9, or 12.9 mg/kg for low-, mid-, or high-dose females. Clinical findings noted during the study were limited to increased incidences of tissue masses on the head, over the dorsum, and in the ventral posterior area.

TABLE 7
Mean Body Weights of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride

Week on Study	0 ppm		30 ppm			70 ppm			150 ppm		
	Av. Wt. (g)	Number Weighed	Av. Wt. (g)	Wt. (% of controls)	Number Weighed	Av. Wt. (g)	Wt. (% of controls)	Number Weighed	Av. Wt. (g)	Wt. (% of controls)	Number Weighed
1	141	70	141	100	45	142	101	75	143	102	70
2	173	70	174	100	45	177	102	75	171	99	70
3	214	70	212	99	45	207	97	74	201	94	70
4	236	70	230	97	45	233	99	74	221	94	70
5	262	70	254	97	45	255	97	74	239	91	70
6	278	70	272	98	45	273	98	74	253	91	70
7	292	70	289	99	45	288	99	74	266	91	70
8	305	70	301	99	12	304	100	74	277	91	70
9	320	70	316	99	45	316	99	74	287	90	70
10	327	70	324	99	45	328	100	74	298	91	70
11	345	70	342	99	45	335	97	74	305	89	70
12	352	70	345	98	45	340	97	74	314	89	70
13	354	70	351	99	45	346	98	74	317	90	70
14	362	70	358	99	45	363	100	74	322	89	70
15	365	70	364	100	45	366	100	74	332	91	70
17	380	70	376	99	45	372	98	74	337	89	70
21	401	70	399	100	45	388	97	74	352	88	70
25	412	70	410	100	45	401	97	74	362	88	70
29	423	70	413	98	45	405	96	74	361	86	70
33	436	70	426	98	45	418	96	73	368	84	67
37	447	70	432	97	44	426	95	72	368	82	64
41	447	60	440	94	44	423	95	72	356	80	44
45	459	60	449	98	44	430	94	67	342	75	34
49	454	60	433	98	44	432	95	67	319	70	27
53	453	60	443	98	43	423	93	64	332	73	6
57	447	60	432	97	42	408	92	57	–	–	0
Mean for Weeks											
1-14	283		279	99		279	99		258	92	
15-57	427		418	98		408	96		319	76	

TABLE 8
Mean Body Weights of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride

Week on Study	0 ppm Av. Wt. (g)	0 ppm Number Weighed	30 ppm Av. Wt. (g)	30 ppm Wt. (% of controls)	30 ppm Number Weighed	70 ppm Av. Wt. (g)	70 ppm Wt. (% of controls)	70 ppm Number Weighed	150 ppm Av. Wt. (g)	150 ppm Wt. (% of controls)	150 ppm Number Weighed
1	114	70	113	100	45	114	100	75	116	102	70
2	130	70	130	100	45	130	100	75	129	99	70
3	145	70	144	99	45	140	96	75	139	95	70
4	154	70	152[a]	99	45	149	96	75	146	94	70
5	164	70	160	98	45	156	96	75	153	94	70
6	173	70	169	98	45	165	96	75	160	92	70
7	179	70	174	97	45	170	95	75	165	92	70
8	184	70	176	96	45	174	94	75	168	92	70
9	188	70	183	97	45	176	94	75	173	92	70
10	193	70	186	96	45	181	94	75	176	91	70
11	196	70	191	98	45	183	94	75	178	91	70
12	198	70	191	97	45	185	94	75	182	92	70
13	199	70	194	97	45	187	94	75	182	91	70
14	203	70	195	96	45	195	96	75	184	90	70
15	207	70	197	95	45	195	94	75	189	91	70
17	210	70	205	97	45	196	93	75	190	90	70
21	217	70	211	97	45	203[b]	94	75	196	90	70
25	225	70	218	97	45	209	93	74	200	89	69
29	228	70	222	97	45	212	93	73	203	89	69
33	238	70	228	96	45	218	92	73	206	87	69
37	247	70	236	96	45	220	89	72	205	83	66
41	251	60	243	97	45	223	89	71	211	84	51
45	264	60	252	96	45	223	85	70	209	79	46
49	271	60	263	97	45	228	84	64	211	78	41
53	280	60	268	96	44	228	82	55	209	75	30
57	287	59	270	94	40	232	81	42	214	74	16
Mean for Weeks											
1-14	173		168	98		165	96		161	93	
15-57	244		234	96		216	89		204	84	

[a] Mean based on 40 animals. One cage inadvertently not weighed.
[b] Mean based on 70 animals. One cage inadvertently not weighed.

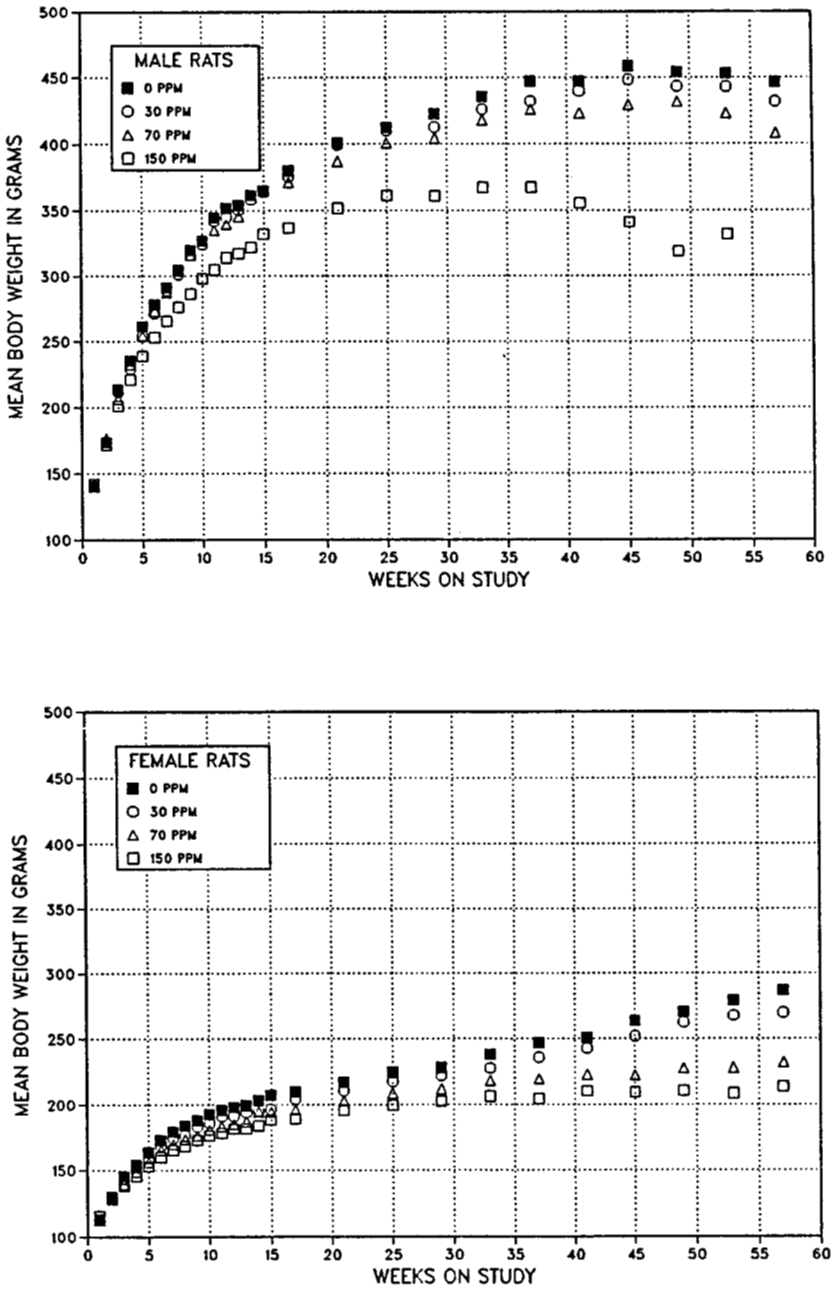


FIGURE 2
Growth Curves for Rats Given Drinking Water Containing 3,3′-Dimethylbenzidine Dihydrochloride for 14 Months

Survival

Estimates of the probabilities of survival for male and female rats given drinking water containing 3,3'-dimethylbenzidine dihydrochloride at the concentrations used in these studies and for controls are shown in Table 9 and in the Kaplan-Meier curves in Figure 3. By week 55, all high-dose males had been found dead or killed moribund; only about 25% of the high-dose females survived to week 56.

TABLE 9
Survival of Rats in the 14-Month Drinking Water Studies of 3,3'-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Male[a]				
Animals initially in study	70	45	75	70
Interim kill (9 months)	10			10
Natural deaths		2	5	15
Moribund kills		2	19	45
Accidental deaths			1	
Animals surviving until study termination	60	41	50	0
Survival P values[b]	<0.001	0.059	<0.001	<0.001
Female[a]				
Animals initially in study	70	45	75	70
Interim kill (9 months)	10			10
Natural deaths		1	6	5
Moribund kills	1	5	37	45
Animals surviving until study termination	59	39	32	10
Survival P values[b]	<0.001	0.049	<0.001	<0.001

[a] First day of termination period: male, 21 August 1984; female, 23 August 1984
[b] 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.

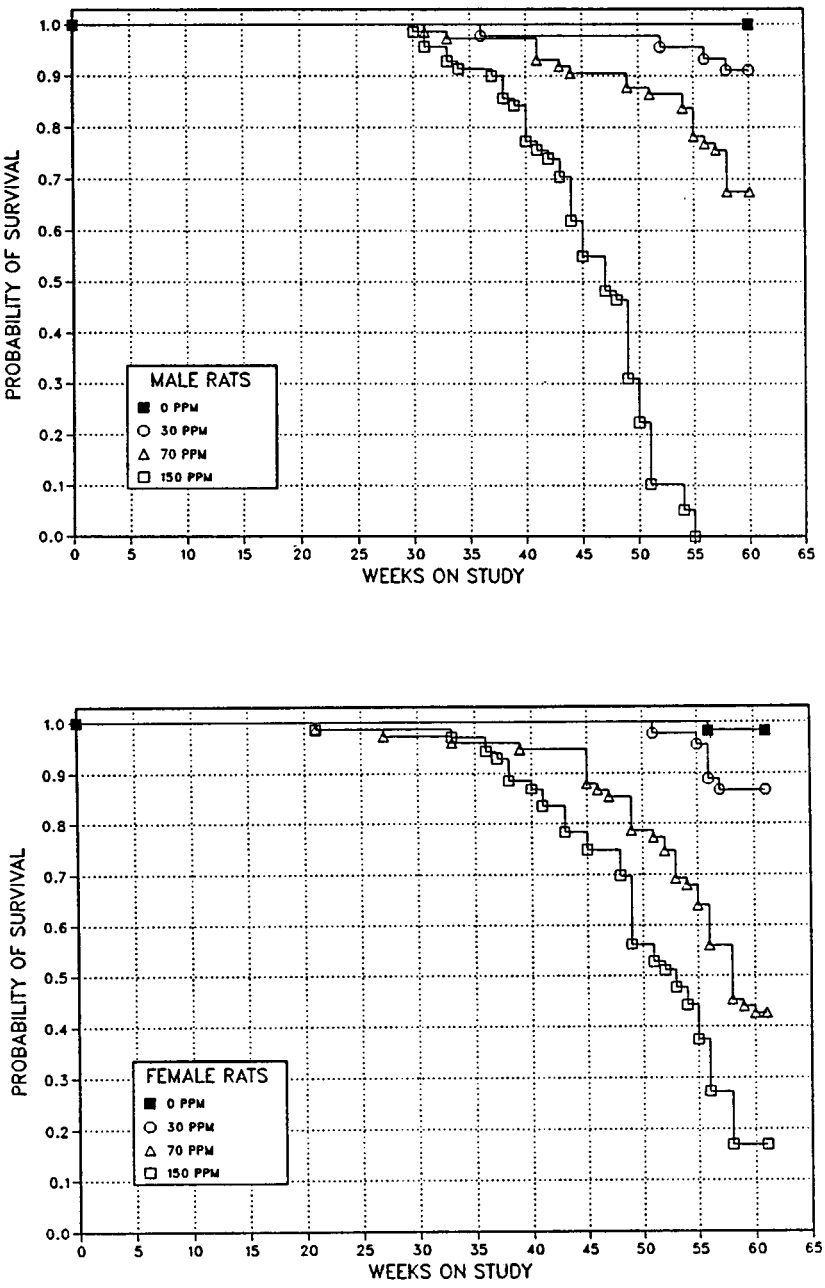


FIGURE 3
Kaplan-Meier Survival Curves for Rats Given Drinking Water Containing 3,3′-Dimethylbenzidine Dihydrochloride for 14 Months

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the skin, Zymbal's gland, preputial and clitoral glands, liver, oral cavity, small intestine, large intestine, mammary gland, lung, mesothelium, brain, adrenal medulla, hematopoietic system, testis, uterus, kidney, parathyroid, heart, glandular stomach, adrenal cortex, spleen, bone marrow, mandibular lymph node, nose, and seminal vesicle.

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

Skin: A variety of epithelial neoplasms of the skin occurred with increased incidence in male and female rats treated with 3,3′-dimethylbenzidine dihydrochloride (Table 10). The increases were often marked in males. A single keratoacanthoma in a male rat was the only epithelial skin neoplasm diagnosed in an untreated animal. The incidence of basal cell adenomas and basal cell carcinomas was increased in treated males and females, and the overall incidence of adenomas in mid- and high-dose males reached 69% and 48%, respectively. The incidence of basal cell adenomas or carcinomas (combined) was significantly increased in all treated males and in mid- and high-dose females. Basal cell adenomas occurred at multiple sites in 47% of mid-dose males and 35% of high-dose males. The incidence of squamous cell papillomas or squamous cell carcinomas (combined) was significantly increased in the mid- and high-dose groups of both sexes. The incidence of keratoacanthomas and sebaceous gland adenomas was significantly increased in mid-and high-dose males. Basal cell neoplasms were locatedbeneath the epidermis and consisted of small polygonal basophilic cells that formed densely packed sheets, branching cords, or solid nodules often containing a central cavity (Figure 4). Adenomas were discrete, circumscribed masses, while carcinomas exhibited local invasion. Many of the basal cell neoplasms contained areas of sebaceous, squamous, or hair follicle differentiation. Squamous cell papillomas were exophytic growths composed of a highly branched fibrovascular core covered by thickened stratified squamous epithelium. Squamous cell carcinomas were plaquelike masses of pleomorphic stratified squamous epithelial cells exhibiting disordered growth and invasion of the underlying dermis. Keratoacanthomas consisted of a central cavity that was often connected to the surface and lined by a thick, highly folded layer of deeply keratinized, stratified squamous epithelium. Sebaceous gland adenomas were composed of multiple glandular structures consisting of nodules of well-differentiated sebaceous cells surrounded by one or more layers of basal cells.

Zymbal's gland: Zymbal's glands are specialized sebaceous glands anterior and ventral to the orifices of the external ear. There was a marked increase in the incidence of Zymbal's gland adenomas and carcinomas in treated male and female rats (Table 11). Some treated rats had bilateral carcinomas of the Zymbal's gland. The incidence of adenomas or carcinomas (combined) was significantly increased in mid- and high-dose males and in all treated females. Carcinomas in some treated rats metastasized to the lung, while others invaded into the brain. Zymbal's glands from some treated animals exhibited nonneoplastic changes, including focal hyperplasia of the glandular cells (hyperplasia, glandular), focal hyperplasia of the stratified squamous epithelium lining the glandular ducts (hyperplasia, squamous), diffuse enlargement of the gland secondary to enlargement of the glandular cells (hypertrophy), and dilation of the glandular ducts (ectasia). There was a morphologic continuum from Zymbal's gland adenoma to carcinoma. Adenomas were discrete masses composed of glandular acini of sebaceous-like cells surrounding ductular structures lined with squamous epithelium. Carcinomas were generally larger and invaded adjacent soft tissue. Neoplastic cells within carcinomas exhibited cellular atypia and disordered growth patterns and formed irregular acinar structures, solid masses, and cords, with a scattering of ductlike structures (Figure 5). Some carcinomas consisted predominantly of sebaceous cells, while others were composed principally of stratified squamous epithelium; some had prominent components of both.

TABLE 10
Skin Tumors in F344/N Rats in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Male				
Keratoacanthoma				
Overall rates	1/60 (0%)	1/45 (2%)	8/75 (11%)	5/60 (8%)
Effective rates[a]	1/60 (2%)	1/44 (2%)	8/67 (12%)	5/27 (19%)
Terminal rates	1/60 (2%)	1/41 (2%)	7/50 (14%)	0/0
Day of first observation	419 (T)	419 (T)	379	338
Life table tests	P<0.001	P=0.674	P=0.010	P<0.001
Cochran-Armitage test[b]	P<0.001			
Fisher exact test[b]		P=0.670	P=0.024	P=0.010
Sebaceous Gland Adenoma				
Overall rates	0/60 (0%)	0/45 (0%)	7/75 (9%)	5/60 (8%)
Effective rates	0/60 (0%)	0/44 (0%)	7/72 (10%)	5/49 (10%)
Terminal rates	0/60 (0%)	0/41 (0%)	5/50 (10%)	0/0
Day of first observation			405	280
Life table tests	P<0.001	–[c]	P=0.006	P=0.001
Cochran-Armitage test	P<0.005			
Fisher exact test		–[c]	P=0.012	P=0.016
Basal Cell Adenoma				
Overall rates	0/60 (0%)	10/45 (22%)	52/75 (69%)	29/60 (48%)
Effective rates	0/60 (0%)	10/44 (23%)	52/72 (72%)	29/45 (64%)
Terminal rates	0/60 (0%)	10/41 (24%)	39/50 (78%)	0/0
Day of first observation		419 (T)	307	281
Life table 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
Basal Cell Carcinoma				
Overall rates	0/60 (0%)	1/45 (2%)	4/75 (5%)	2/60 (3%)
Effective rates	0/60 (0%)	1/44 (2%)	4/68 (6%)	2/43 (5%)
Terminal rates	0/60 (0%)	1/41 (2%)	4/50 (8%)	0/0
Day of first observation		419 (T)	419 (T)	296
Life table tests	P<0.001	P=0.424	P=0.043	P=0.127
Cochran-Armitage test	P=0.121			
Fisher exact test		P=0.423	P=0.076	P=0.172
Basal Cell Adenoma or Carcinoma[d]				
Overall rates	0/60 (0%)	11/45 (24%)	54/75 (72%)	30/60 (50%)
Effective rates	0/60 (0%)	11/44 (25%)	54/72 (75%)	30/45 (67%)
Terminal rates	0/60 (0%)	11/41 (27%)	41/50 (82%)	0/0
Day of first observation		419 (T)	307	281
Life table 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 10
Skin Tumors in F344/N Rats in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Squamous Cell Papilloma				
Overall rates	0/60 (0%)	0/45 (0%)	8/75 (11%)	15/60 (25%)
Effective rates	0/60 (0%)	0/45 (0%)	8/72 (11%)	15/55 (27%)
Terminal rates	0/60 (0%)	0/41 (0%)	6/50 (12%)	0/0
Day of first observation			405	238
Life table tests	P<0.001	–[c]	P=0.003	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		–[c]	P=0.006	P<0.001
Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	2/45 (4%)	10/75 (13%)	13/60 (22%)
Effective rates	0/60 (0%)	2/45 (4%)	10/74 (14%)	13/59 (22%)
Terminal rates	0/60 (0%)	1/41 (2%)	9/50 (18%)	0/0
Day of first observation		391	406	211
Life table tests	P<0.001	P=0.165	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P=0.002	P<0.001
Squamous Cell Papilloma or Carcinoma[e]				
Overall rates	0/60 (0%)	2/45 (4%)	17/75 (23%)	27/60 (45%)
Effective rates	0/60 (0%)	2/45 (4%)	17/74 (23%)	27/59 (46%)
Terminal rates	0/60 (0%)	1/41 (2%)	14/50 (28%)	0/0
Day of first observation		391	405	211
Life table tests	P<0.001	P=0.165	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P=0.001	P<0.001
Female				
Keratoacanthoma				
Overall rates	0/60 (0%)	0/45 (0%)	0/75 (0%)	1/60 (2%)
Sebaceous Gland Adenoma				
Overall rates	0/60 (0%)	0/45 (0%)	1/75 (1%)	1/60 (2%)
Basal Cell Adenoma				
Overall rates	0/60 (0%)	3/45 (7%)	5/75 (7%)	5/60 (8%)
Effective rates	0/60 (0%)	3/45 (7%)	5/64 (8%)	5/41 (12%)
Terminal rates	0/59 (0%)	3/39 (8%)	3/32 (9%)	2/10 (20%)
Day of first observation		421 (T)	338	370
Life table tests	P<0.001	P=0.060	P=0.013	P<0.001
Cochran-Armitage test	P=0.014			
Fisher exact test		P=0.076	P=0.034	P=0.009
Basal Cell Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	5/75 (7%)	4/60 (7%)
Effective rates	0/60 (0%)	0/45 (0%)	5/69 (7%)	4/46 (9%)
Terminal rates	0/59 (0%)	0/39 (0%)	1/32 (3%)	0/10 (0%)
Day of first observation			315	336
Life table tests	P<0.001	–[c]	P=0.015	P=0.010
Cochran-Armitage test	P=0.009			
Fisher exact test		–[c]	P=0.041	P=0.033

TABLE 10
Skin Tumors in F344/N Rats in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Basal Cell Adenoma or Carcinoma[f]				
Overall rates	0/60 (0%)	3/45 (7%)	10/75 (13%)	9/60 (15%)
Effective rates	0/60 (0%)	3/45 (7%)	10/69 (14%)	9/46 (20%)
Terminal rates	0/59 (0%)	3/39 (8%)	4/32 (13%)	2/10 (20%)
Day of first observation		421 (T)	315	336
Life table tests	P<0.001	P=0.060	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.076	P=0.001	P<0.001
Squamous Cell Papilloma				
Overall rates	0/60 (0%)	1/45 (2%)	6/75 (8%)	5/60 (8%)
Effective rates	0/60 (0%)	1/45 (2%)	6/72 (8%)	5/55 (9%)
Terminal rates	0/59 (0%)	1/39 (3%)	4/32 (13%)	0/10 (0%)
Day of first observation		421 (T)	391	264
Life table tests	P<0.001	P=0.417	P=0.003	P=0.002
Cochran-Armitage test	P=0.015			
Fisher exact test		P=0.429	P=0.024	P=0.023
Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	2/45 (4%)	4/75 (5%)	7/60 (12%)
Effective rates	0/60 (0%)	2/45 (4%)	4/64 (6%)	7/41 (17%)
Terminal rates	0/59 (0%)	2/39 (5%)	3/32 (9%)	3/10 (30%)
Day of first observation		421 (T)	406	338
Life table tests	P<0.001	P=0.153	P=0.016	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P=0.068	P=0.001
Squamous Cell Papilloma or Carcinoma[g]				
Overall rates	0/60 (0%)	3/45 (7%)	9/75 (12%)	12/60 (20%)
Effective rates	0/60 (0%)	3/45 (7%)	9/72 (13%)	12/55 (22%)
Terminal rates	0/59 (0%)	3/39 (8%)	6/32 (19%)	3/10 (30%)
Day of first observation		421 (T)	391	264
Life table tests	P<0.001	P=0.060	P<0.001	P<0.001
Cochran-Armitage trend test	P<0.001			
Fisher exact test		P=0.076	P=0.003	P<0.001

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the first occurrence of this tumor type in any of the groups
[b] Based on effective rates
[c] No tumors in dosed group or control group; statistical test not performed
[d] 2-year historical incidence for untreated control groups at study laboratory (mean): 2/100 (2%); historical incidence for untreated control groups in NTP studies (mean ± SD): 21/1,596 (1.3% ± 1.9%)
[e] 2-year historical incidence for untreated control groups at study laboratory (mean): 3/100 (3%); historical incidence for untreated control groups in NTP studies (mean ± SD): 29/1,596 (1.8% ± 1.6%)
[f] 2-year historical incidence for untreated control groups at study laboratory (mean): 0/100 (0%); historical incidence for untreated control groups in NTP studies (mean ± SD): 6/1,643 (0.4% ± 0.7%)
[g] 2-year historical incidence for untreated control groups at study laboratory (mean): 0/100 (0%); historical incidence for untreated control groups in NTP studies (mean ± SD): 7/1,643 (0.4% ± 0.8%)

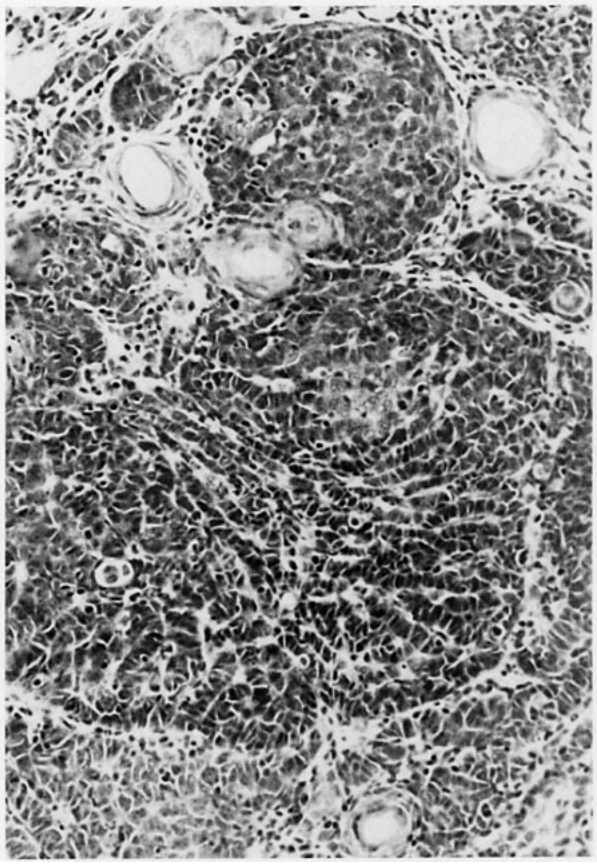


FIGURE 4. Basal cell carcinoma of the skin in a male F344/N rat administered 70 ppm 3,3′-dimethylbenzidine dihydrochloride in drinking water for 14 months. The neoplasm consists of cords and solid clusters of pleomorphic basal cells. At top of figure multiple clusters of neoplastic cells are seen invading the adjacent connective tissue. (×175)

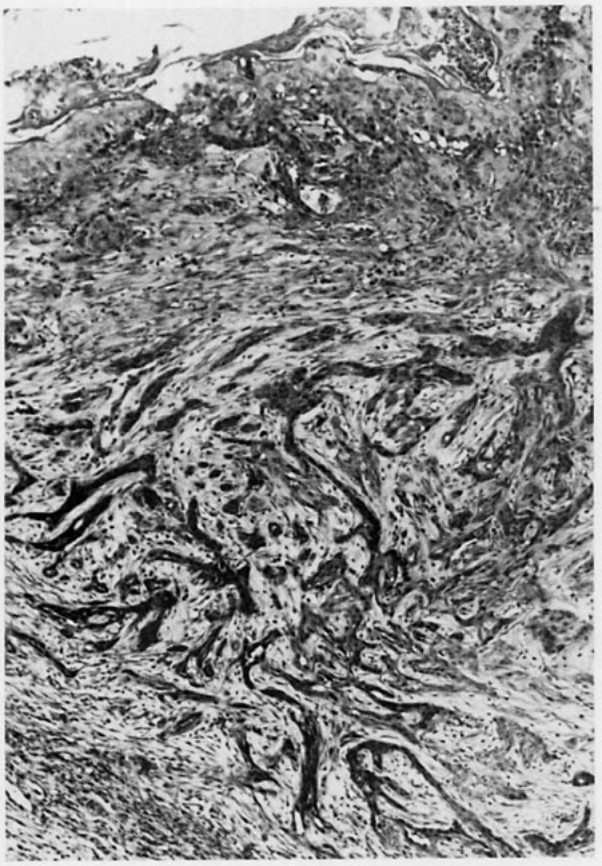


FIGURE 5. Zymbal's gland carcinoma in a female F344/N rat administered 150 ppm 3,3′-dimethylbenzidine dihydrochloride in drinking water for 14 months. This carcinoma consists of stratified squamous epithelium that forms irregular cords and deeply invades the underlying connective tissue. (×75)

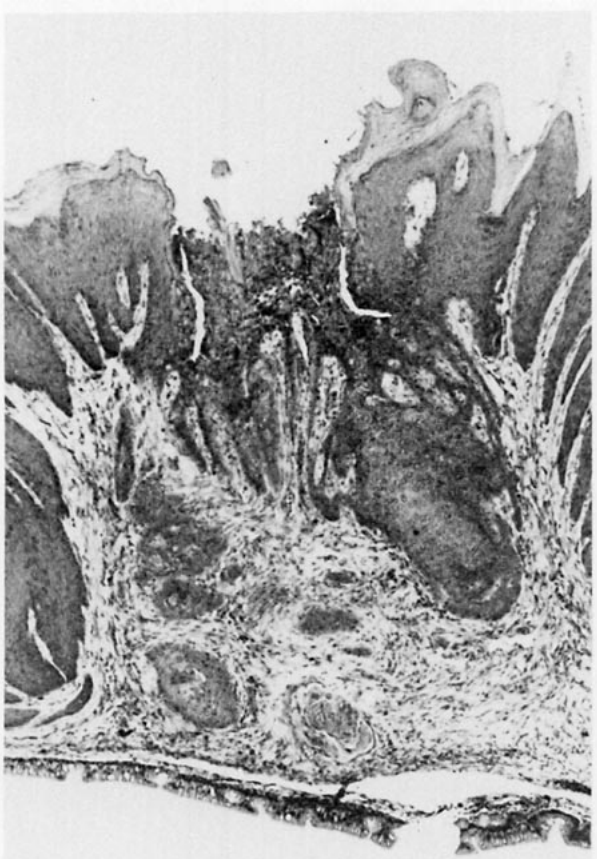


FIGURE 6. Squamous cell carcinoma of the posterior oral cavity in a male F344/N rat administered 150 ppm 3,3′-dimethylbenzidine dihydrochloride in drinking water for 14 months. Cords and clusters of neoplastic epithelial cells have deeply invaded the underlying connective tissue. The layer of respiratory epithelium lining the nasopharynx appears at the bottom of figure. (×70)

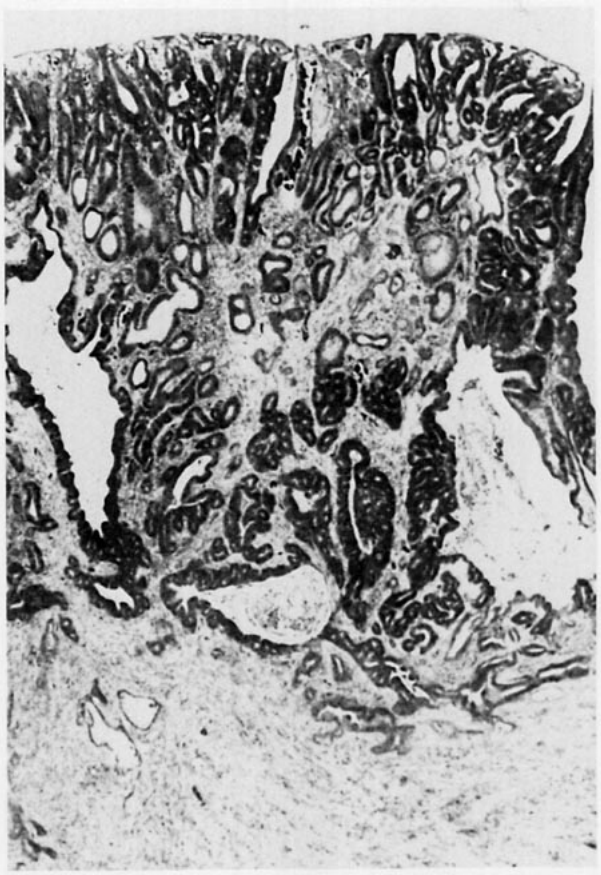


FIGURE 7. Adenocarcinoma of the mucuosal epithelium of the colon in a male F344/N rat administered 150 ppm 3,3′-dimethylbenzidine dihydrochloride in drinking water for 14 months. Multiple irregular glandular structures composed of neoplasitc epithelial cells have invaded the underlying submucosa, causing the mucosa to appear substantially thicker than normal. Some of the glands are dilated and contain mucus and debris. (×35)

TABLE 11
Zymbal's Gland Lesions in F344/N Rats in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Male				
Hyperplasia				
Overall rates	0/59 (0%)	0/45 (0%)	1/75 (1%)	8/59 (14%)
Adenoma				
Overall rates	1/60 (2%)	1/45 (2%)	13/75 (17%)	16/60 (27%)
Effective rates[a]	1/60 (2%)	1/44 (2%)	13/72 (18%)	16/54 (30%)
Terminal rates	1/60 (2%)	1/41 (2%)	10/50 (20%)	0/0
Day of first observation	419 (T)	419 (T)	378	254
Life table tests	P<0.001	P=0.674	P<0.001	P<0.001
Cochran-Armitage test[b]	P<0.001			
Fisher exact test[b]		P=0.670	P=0.002	P<0.001
Carcinoma				
Overall rates	0/60 (0%)	2/45 (4%)	21/75 (28%)	23/60 (38%)
Effective rates	0/60 (0%)	2/45 (4%)	21/74 (28%)	23/60 (38%)
Terminal rates	0/59 (0%)	0/41 (0%)	6/50 (12%)	0/0
Day of first observation		359	229	209
Life table tests	P<0.001	P=0.170	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P<0.001	P<0.001
Adenoma or Carcinoma[c]				
Overall rates	1/60 (2%)	3/45 (7%)	32/75 (43%)	36/60 (60%)
Effective rates	1/60 (2%)	3/45 (7%)	32/74 (43%)	36/60 (60%)
Terminal rates	1/59 (2%)	1/41 (2%)	15/50 (30%)	0/0
Day of first observation	419 (T)	359	229	209
Life table tests	P<0.001	P=0.192	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.209	P<0.001	P<0.001
Female				
Hyperplasia				
Overall rates	0/60 (0%)	4/44 (9%)	7/73 (9%)	2/60 (3%)
Adenoma				
Overall rates	0/60 (0%)	4/45 (9%)	11/75 (15%)	12/60 (20%)
Effective rates	0/60 (0%)	4/45 (9%)	11/72 (15%)	12/57 (21%)
Terminal rates	0/59 (0%)	4/39 (10%)	5/32 (16%)	3/10 (30%)
Day of first observation		421 (T)	338	251
Life table tests	P<0.001	P=0.024	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.031	P<0.001	P<0.001

TABLE 11
Zymbal's Gland Lesions in F344/N Rats in the 14-Month Drinking Water Studies of 3,3'-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Carcinoma				
Overall rates	0/60 (0%)	2/45 (4%)	22/75 (29%)	35/60 (58%)
Effective rates	0/60 (0%)	2/45 (4%)	22/74 (30%)	35/59 (59%)
Terminal rates	0/59 (0%)	0/39 (0%)	1/32 (3%)	3/10 (30%)
Day of first observation		357	184	229
Life table tests	P<0.001	P=0.176	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P<0.001	P<0.001
Adenoma or Carcinoma[d]				
Overall rates	0/57 (0%)	6/45 (13%)	32/75 (43%)	42/60 (70%)
Effective rates	0/60 (0%)	6/4[illegible] (13%)	32/74 (43%)	42/59 (71%)
Terminal rates	0/59 (0%)	4/39 (10%)	6/32 (19%)	5/10 (50%)
Day of first observation		357	184	229
Life table tests	P<0.001	P=0.[illegible]	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.005	P<0.001	P<0.001

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the first occurrence of this tumor type in any of the groups
[b] Based on effective rates
[c] 2-year historical incidence for untreated control groups at study laboratory (mean): 1/100 (1%); historical incidence for untreated control groups in NTP studies (mean ± SD): 19/1,596 (1.2% ± 1.9%)
[d] 2-year historical incidence for untreated control groups at study laboratory (mean): 1/100 (1%); historical incidence for untreated control groups in NTP studies (mean ± SD): 14/1,643 (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 adjacent to the penis. There was a marked increase in the incidence of clitoral gland adenomas and carcinomas in treated females (Table 12) and a moderate increase in the incidence of preputial gland adenomas in treated males (Table 13). A few female rats developed bilateral clitoral gland carcinomas. The increase in incidence of adenomas, carcinomas, and adenomas or carcinomas (combined) was significant in all treated female groups. The increase in preputial gland neoplasms was significant only in high-dose males. Adenomas were discrete expansile masses exhibiting some loss of normal acinar architecture. Neoplastic cells were well differentiated and arranged in solid clusters with a scattering of ductlike structures containing debris. Carcinomas were poorly defined masses composed of disorganized sheets of pleomorphic cells that sometimes invaded the adjacent tissue. Carcinomas commonly contained areas of necrosis and exhibited greater cellular atypia and disordered growth than adenomas.

TABLE 12
Clitoral Gland Lesions in Female F344/N Rats in the 14-Month Drinking Water Study of 3,3'-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Hyperplasia				
Overall rates	0/60 (0%)	1/45 (2%)	4/75 (5%)	0/59 (0%)
Adenoma				
Overall rates	0/60 (0%)	9/45 (20%)	32/75 (43%)	17/59 (29%)
Effective rates[a]	0/60 (0%)	9/45 (20%)	32/73 (44%)	17/58 (29%)
Terminal rates	0/59 (0%)	5/39 (13%)	14/32 (44%)	5/10 (50%)
Day of first observation		391	229	296
Life table tests	P<0.001	P<0.001	P<0.001	P<0.001
Cochran-Armitage test[b]	P<0.001			
Fisher exact test[b]		P<0.001	P<0.001	P<0.001
Carcinoma				
Overall rates	0/60 (0%)	5/45 (11%)	11/75 (15%)	18/59 (29%)
Effective rates	0/60 (0%)	5/45 (11%)	11/72 (15%)	18/55 (33%)
Terminal rates	0/59 (0%)	5/39 (13%)	3/32 (9%)	3/10 (30%)
Day of first observation		421 (T)	315	254
Life table tests	P<0.001	P=0.010	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.013	P<0.001	P<0.001
Adenoma or Carcinoma[c]				
Overall rates	0/60 (0%)	14/45 (31%)	42/75 (56%)	32/59 (54%)
Effective rates	0/60 (0%)	14/45 (31%)	42/73 (58%)	32/58 (55%)
Terminal rates	0/59 (0%)	10/39 (26%)	16/32 (50%)	7/10 (70%)
Day of first observation		391	229	254
Life table 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

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the first occurrence of this tumor type in any of the groups
[b] Based on effective rates
[c] Historical incidence for untreated control groups at the study laboratory (mean): 8/100 (8%); historical incidence for untreated control groups in NTP studies (mean ± SD): 115/1,643 (7.0 ± 4.8%)

TABLE 13
Preputial Gland Lesions in Male F344/N Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Hyperplasia				
Overall rates	0/60 (0%)	0/45 (0%)	1/75 (1%)	5/60 (8%)
Adenoma				
Overall rates	2/60 (3%)	4/45 (9%)	4/75 (5%)	8/60 (13%)
Effective rates[a]	2/60 (3%)	4/44 (9%)	4/72 (6%)	8/49 (16%)
Terminal rates	2/60 (3%)	4/41 (10%)	4/50 (8%)	0/0
Day of first observation	419 (T)	419 (T)	419 (T)	280
Life table tests	P<0.001	P=0.182	P=0.258	P<0.001
Cochran-Armitage test[b]	P=0.018			
Fisher exact test[b]		P=0.206	P=0.430	P=0.022
Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	2/75 (3%)	1/60 (2%)
Adenoma or Carcinoma[c]				
Overall rates	2/60 (3%)	4/45 (9%)	6/75 (8%)	9/60 (15%)
Effective rates	2/60 (3%)	4/44 (9%)	6/72 (8%)	9/49 (18%)
Terminal rates	2/60 (3%)	4/41 (10%)	6/50 (12%)	0/0
Day of first observation	419 (T)	419 (T)	419 (T)	280
Life table tests	P<0.001	P=0.182	P=0.086	P<0.001
Cochran-Armitage test	P=0.008			
Fisher exact test		P=0.206	P=0.205	P=0.011

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the first occurrence of this tumor type in any of the four groups
[b] Based on effective rates
[c] 2-year historical incidence for untreated control groups at study laboratory (mean): 5/100 (5%); historical incidence of untreated control groups in NTP studies (mean ± SD): 117/1,596 (7.3% ± 5.2%)

Liver: A variety of neoplastic and nonneoplastic lesions occurred with increased incidence in male and female rats treated with 3,3′-dimethylbenzidine dihydrochloride (Tables 14 and 15). The incidence of hepatocellular neoplasms was markedly increased in mid- and high-dose males and moderately increased in mid- and high-dose females; no hepatocellular neoplasms occurred in the control or low-dose groups. In treated males there was a marked increase in the incidence of both neoplastic nodules and hepatocellular carcinomas in the mid- and high-dose groups. (Neoplastic nodule was the term used previously for proliferative hepatocellular lesions currently classified as hepatocellular adenoma.) The increase in incidence of neoplastic nodules, hepatocellular carcinomas, and neoplastic nodule or hepatocellular carcinoma (combined) was highly statistically significant. A few males had multiple carcinomas, and many had multiple neoplastic nodules. A single hepatoblastoma occurred in one high-dose male. There was a moderate increase in the incidence of neoplastic nodules in mid- and high-dose females, and one mid-dose and one high-dose female each had a single carcinoma. The incidence of neoplastic nodules or carcinomas (combined) was significantly increased in both the mid- and high-dose female groups. Neoplastic nodules were discrete expansile masses that were larger than hepatic lobules and compressed the adjacent parenchyma. Hepatic plates within the neoplastic nodules were not organized in a normal lobular pattern and often intersected at near right angles with the plates in the adjacent normal liver. Neoplastic hepatocytes exhibited altered staining properties and slight pleomorphism and atypia. Hepatocellular carcinomas were larger than neoplastic nodules and consisted of markedly disorganized hepatocytes that formed solid clusters, glandular structures, or broad trabeculae several cell layers thick. Neoplastic hepatocytes generally showed moderate to marked pleomorphism and atypia. Hepatoblastomas arise within existing carcinomas and are thought to represent a highly undifferentiated form of hepatocellular neoplasm. The hepatoblastoma consisted of irregular clusters and cords of small fusiform cells with scant cytoplasm and deeply basophilic nuclei.

Cystic degeneration, hematopoietic cell proliferation, and foci of cellular alteration (basophilic, eosinophilic, and mixed cell foci) occurred with markedly increased incidence in treated rats of each sex. The incidence of focal and multifocal hepatocellular necrosis was slightly increased in both sexes, and the incidence of fatty change of hepatocytes was slightly increased in high-dose males and mid-dose females. The incidence of bile duct hyperplasia was markedly reduced in both sexes of treated rats as compared with controls; this is probably a reflection of the reduced survival in treated rats. Cystic degeneration is a common degenerative change in the rat liver and consists of focal clusters of variably sized cystic spaces containing granular eosinophilic material or erythrocytes. The increased incidence of hematopoiesis was presumably secondary to inflammation associated with the neoplasms in treated animals. Foci of cellular alteration consisted of poorly demarcated clusters of hepatocytes with altered cytoplasmic staining. Although some were large enough to be seen grossly, many of these foci were smaller than a hepatic lobule and caused minimal or no compression, blending with the adjacent normal parenchyma. Basophilic foci were characterized by cells with basophilic cytoplasm, while eosinophilic foci were composed of cells with cytoplasm that stained more vividly eosinophilic than that of normal hepatocytes. Mixed cell foci consisted of mixtures of cells with eosinophilic cytoplasm and cells with clear cytoplasm.

Oral Cavity (Tongue or Pharynx): Treatment with 3,3′-dimethylbenzidine dihydrochloride resulted in several squamous cell papillomas and carcinomas of the tongue and pharynx in male and female rats (Table 16). No squamous cell neoplasms of the oral cavity occurred in untreated rats of either sex. The incidence of squamous cell papillomas or carcinomas (combined) was significantly increased in the high-dose male and mid- and high-dose female groups. Papillomas were exophytic masses arising from the oral mucosal surface and extending into the oral cavity. They consisted of a stalk-like, highly branched core of fibrous tissue covered by a thickened layer of stratified squamous epithelium. Squamous cell carcinomas were flat, broad-based lesions of the oral epithelium consisting of disorganized clusters and cords of pleomorphic squamous epithelial cells invading the underlying submucosa (Figure 6). Invasion was often accompanied by fibrous tissue proliferation and inflammation.

TABLE 14
Liver Tumors in F344/N Rats in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Male				
Neoplastic Nodule[a]				
Overall rates	0/60 (0%)	0/45 (0%)	29/75 (39%)	26/60 (43%)
Effective rates[b]	0/60 (0%)	0/44 (0%)	29/72 (40%)	26/49 (53%)
Terminal rates	0/60 (0%)	0/41 (0%)	23/50 (46%)	0/0
Day of first observation			393	280
Cochran-Armitage test[c]	P<0.001			
Fisher exact test[c]		–[d]	P<0.001	P<0.001
Hepatocellular Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	12/75 (16%)	12/60 (20%)
Effective rates	0/60 (0%)	0/45 (0%)	12/72 (17%)	12/55 (22%)
Terminal rates	0/60 (0%)	0/41 (0%)	11/50 (22%)	0/0
Day of first observation			379	238
Cochran-Armitage test	P<0.001			
Fisher exact test		–	P<0.001	P<0.001
Neoplastic Nodule or Hepatocellular Carcinoma[e]				
Overall rates	0/60 (0%)	0/45 (0%)	35/75 (47%)	33/60 (55%)
Effective rates	0/60 (0%)	0/45 (0%)	35/72 (49%)	33/55 (60%)
Terminal rates	0/60 (0%)	0/41 (0%)	28/50 (56%)	0/0
Day of first observation			379	238
Cochran-Armitage test	P<0.001			
Fisher exact test		–	P<0.001	P<0.001
Female				
Neoplastic Nodule				
Overall rates	0/60 (0%)	0/45 (0%)	7/74 (9%)	3/60 (5%)
Effective rates	0/60 (0%)	0/45 (0%)	7/58 (12%)	3/36 (8%)
Terminal rates	0/59 (0%)	0/39 (0%)	6/32 (19%)	0/10 (0%)
Day of first observation			378	343
Cochran-Armitage test	P=0.014			
Fisher exact test		–	P=0.006	P=0.050
Hepatocellular Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	1/74 (1%)	1/60 (2%)
Neoplastic Nodule[b] **or Hepatocellular Carcinoma**[f]				
Overall rates	0/60 (0%)	0/45 (0%)	7/74 (9%)	4/60 (7%)
Effective rates	0/60 (0%)	0/45 (0%)	7/58 (12%)	4/36 (11%)
Terminal rates	0/59 (0%)	0/39 (0%)	6/32 (19%)	0/10 (0%)
Day of first observation			378	343
Cochran-Armitage test	P=0.004			
Fisher exact test		–	P=0.006	P=0.018

(T)Terminal sacrifice
[a] Term used previously for lesions currently classified as hepatocellular adenoma.
[b] Number of tumor-bearing animals/effective number of animals, i.e., number of animals alive at the first occurrence of tumors in any of the groups
[c] Based on effective rates
[d] No tumors in dosed group or control group; no statistics performed
[e] 2-year historical incidence for untreated control groups at study laboratory (mean): 7/100 (7%); historical incidence for untreated control groups in NTP studies (mean ± SD): 78/1,591 (4.9% ± 4.3%)
[f] 2-year historical incidence for untreated control groups at study laboratory (mean): 2/100 (2%); historical incidence for untreated control groups in NTP studies (mean ± SD): 37/1,643 (2.3% ± 2.7%)

TABLE 15
Numbers of F344/N Rats with Selected Nonneoplastic Liver Lesions in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

Lesion	Male				Female			
	0 ppm	30 ppm	70 ppm	150 ppm	0 ppm	30 ppm	70 ppm	150 ppm
Number examined	60	45	75	60	60	45	74	60
Basophilic focus	1	31 ***	54 ***	27 ***	0	13 ***	11 ***	3
Eosinophilic focus	0	0	57 ***	53 ***	0	7 **	57 ***	38***
Mixed cell focus	0	37 ***	54 ***	30 ***	0	34 ***	49 ***	32***
Fatty change	1	2	1	7 *	0	0	4	2
Hematopoietic cell proliferation	0	2	27 ***	15 ***	0	7 **	19 ***	8**
Cystic degeneration	0	24 ***	67 ***	51 ***	0	3	12 ***	11***
Focal or multifocal necrosis	3	4	10	5	0	3	7 *	2

* Significantly different ($P \leq 0.05$) from the control group by Fisher exact test; based on effective rates
** $P \leq 0.01$
*** $P \leq 0.001$

TABLE 16
Squamous Cell Tumors of the Oral Cavity in F344/N Rats in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Male				
Squamous Cell Papilloma				
Overall rates	0/60 (0%)	0/45 (0%)	3/75 (4%)	2/60 (3%)
Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	1/75 (1%)	3/60 (5%)
Squamous Cell Papilloma or Carcinoma[d]				
Overall rates	0/60 (0%)	0/45 (0%)	4/75 (5%)	5/60 (8%)
Effective rates[a]	0/60 (0%)	0/44 (0%)	4/67 (6%)	5/32 (16%)
Terminal rates	0/60 (0%)	0/41 (0%)	3/50 (6%)	0/0 (0%)
Day of first observation			341	324
Cochran-Armitage test[b]	P<0.001			
Fisher exact test[b]		–[c]	P=0.074	P=0.004
Female				
Squamous Cell Papilloma				
Overall rates	0/60 (0%)	3/45 (7%)	7/75 (9%)	9/60 (15%)
Effective rates	0/60 (0%)	3/45 (7%)	7/73 (10%)	9/59 (15%)
Terminal rates	0/59 (0%)	3/39 (8%)	3/32 (9%)	3/10 (30%)
Day of first observation		421 (T)	363	229
Cochran-Armitage test	P=0.002			
Fisher exact test		P=0.076	P=0.013	P=0.001
Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	1/45 (2%)	2/75 (3%)	4/60 (7%)
Effective rates	0/60 (0%)	1/45 (2%)	2/64 (3%)	4/41 (10%)
Terminal rates	0/59 (0%)	1/39 (3%)	2/32 (6%)	1/10 (10%)
Day of first observation		421 (T)	421 (T)	338
Cochran-Armitage test	P=0.008			
Fisher exact test		P=0.429	P=0.264	P=0.025
Squamous Cell Papilloma or Carcinoma[d]				
Overall rates	0/60 (0%)	3/45 (7%)	9/75 (12%)	13/60 (22%)
Effective rates	0/60 (0%)	3/45 (7%)	9/73 (12%)	13/59 (22%)
Terminal rates	0/59 (0%)	3/39 (8%)	5/32 (16%)	4/10 (40%)
Day of first observation		421 (T)	363	229
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.076	P=0.004	P<0.001

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the first occurrence of this tumor type in any of the groups
[b] Based on effective rates
[c] No tumors in dosed group or control group; statistical test not performed
[d] Details for the incidence of these neoplasms in the tongue and pharynx are presented in Tables A1 (males) and B1 (females). Historical incidence in untreated control groups in NTP studies: 7/1,596 for males and 4/1,643 for females.

Small Intestine (Duodenum, Jejunum, or Ileum): Adenocarcinomas and adenomatous polyps of the small intestine mucosa occur rarely in untreated F344/N rats. Several of these neoplasms were found in treated male and female rats in these studies (Table 17). All neoplasms occurred in mid- and high-dose rats, with the exception of a single adenomatous polyp in a low-dose female. The incidence of adenomatous polyps or adenocarcinomas (combined) was significantly increased in the high-dose male and female groups. Adenocarcinomas were poorly demarcated masses that invaded the intestinal wall. They consisted of irregular, variably sized clusters and glandular structures of moderately to poorly differentiated columnar cells, often surrounded by proliferating fibrous tissue containing inflammatory cells. Some adenocarcinomas contained mucus secreting cells that formed large cystic spaces filled with mucus (cystic mucinous adenocarcinoma). Adenomatous polyps were pedunculated masses that projected into the intestinal lumen. They consisted of a stalk-like core of fibrous tissue covered by numerous glandular structures lined by a single layer of moderately well-differentiated tall columnar cells with round nuclei and abundant basophilic cytoplasm.

Large Intestine (Cecum, Colon, or Rectum): Adenocarcinomas and adenomatous polyps of the large intestine mucosa are rarely seen in untreated F344/N rats. Several of these neoplasms occurred in treated male and female rats in these studies (Table 18). All neoplasms occurred in mid- or high-dose animals, except for a single adenomatous polyp in a low-dose female. Two high-dose males had multiple polyps. The incidence of adenomatous polyps or adenocarcinomas (combined) was significantly increased in the high-dose male and female groups.

The histologic appearance of adenocarcinomas (Figure 7) and adenomatous polyps of the large intestine was similar to that of the small intestine.

TABLE 17
Tumors of the Small Intestine in F344/N Rats in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Male				
Adenomatous Polyp				
Overall rates	0/60 (0%)	0/45 (0%)	1/75 (1%)	1/60 (2%)
Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	3/75 (4%)	8/60 (13%)
Effective rates[a]	0/60 (0%)	0/45 (0%)	3/74 (4%)	8/59 (14%)
Terminal rates	0/60 (0%)	0/41 (0%)	3/50 (6%)	0/0 (0%)
Day of first observation			419 (T)	211
Cochran-Armitage test[b]	P<0.001			
Fisher exact test[b]		–[c]	P=0.165	P=0.003
Adenomatous Polyp or Adenocarcinoma[d]				
Overall rates	0/60 (0%)	0/45 (0%)	4/75 (5%)	8/60 (13%)
Effective rates	0/60 (0%)	0/45 (0%)	4/74 (5%)	8/59 (14%)
Terminal rates	0/60 (0%)	0/41 (0%)	3/50 (6%)	0/0 (0%)
Day of first observation			379	211
Cochran-Armitage test	P<0.001			
Fisher exact test		–	P=0.090	P=0.003
Female				
Adenomatous Polyp				
Overall rates	0/60 (0%)	1/45 (2%)	1/75 (1%)	0/60 (0%)
Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	2/75 (3%)	5/60 (8%)
Effective rates	0/60 (0%)	0/45 (0%)	2/72 (3%)	5/57 (9%)
Terminal rates	0/59 (0%)	0/39 (0%)	0/32 (0%)	0/10 (0%)
Day of first observation			309	251
Cochran-Armitage test	P=0.003			
Fisher exact test		–	P=0.296	P=0.025
Adenomatous Polyp or Adenocarcinoma[e]				
Overall rates	0/60 (0%)	1/45 (2%)	3/75 (5%)	5/60 (8%)
Effective rates	0/60 (0%)	1/45 (2%)	3/72 (4%)	5/57 (9%)
Terminal rates	0/59 (0%)	0/39 (0%)	1/32 (3%)	0/10 (0%)
Day of first observation		391	309	251
Cochran-Armitage test	P=0.011			
Fisher exact test		P=0.429	P=0.159	P=0.025

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the first occurrence of this tumor type in any of the groups
[b] Based on effective rates
[c] No tumors in dosed group or control group; statistical test not performed
[d] 2-year historical incidence for untreated control groups at study laboratory (mean): 1/97 (1%); historical incidence for untreated control groups in NTP studies (mean ± SD): 5/1,557 (0.3± 0.7%)
[e] 2-year historical incidence for untreated control groups at study laboratory (mean): 0/99; historical incidence for untreated control groups in NTP studies (mean ± SD): 0/1,611

TABLE 18
Tumors of the Large Intestine in F344/N Rats in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Male				
Adenomatous Polyp				
Overall rates	0/60 (0%)	0/45 (0%)	6/75 (8%)	9/60 (15%)
Effective rates[a]	0/60 (0%)	0/44 (0%)	6/67 (9%)	9/38 (24%)
Terminal rates	0/60 (0%)	0/41 (0%)	5/50 (10%)	0/0 (0%)
Day of first observation			384	308
Cochran-Armitage test[b]	P<0.001			
Fisher exact test[b]		–[c]	P=0.019	P<0.001
Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	0/75 (0%)	7/60 (12%)
Effective rates	0/60 (0%)	0/45 (0%)	0/67 (0%)	7/36 (19%)
Terminal rates	0/60 (0%)	0/41 (0%)	0/50 (0%)	0/0 (0%)
Day of first observation				309
Cochran-Armitage test	P=0.001			
Fisher exact test		–	–	P=0.001
Adenomatous Polyp or Adenocarcinoma[d]				
Overall rates	0/60 (0%)	0/45 (0%)	6/75 (8%)	15/60 (25%)
Effective rates	0/60 (0%)	0/45 (0%)	6/67 (9%)	15/38 (39%)
Terminal rates	0/60 (0%)	0/41 (0%)	5/50 (10%)	0/0 (0%)
Day of first observation			384	308
Cochran-Armitage test	P<0.001			
Fisher exact test		–	P=0.019	P<0.001
Female				
Adenomatous Polyp				
Overall rates	0/60 (0%)	1/45 (2%)	6/75 (8%)	4/60 (7%)
Effective rates	0/60 (0%)	1/45 (2%)	6/70 (9%)	4/46 (9%)
Terminal rates	0/59 (0%)	1/39 (3%)	4/32 (13%)	1/10 (0%)
Day of first observation		421 (T)	310	355
Cochran-Armitage test	P=0.020			
Fisher exact test		P=0.429	P=0.022	P=0.033
Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	1/75 (1%)	1/60 (2%)
Adenomatous Polyp or Adenocarcinoma[e]				
Overall rates	0/60 (0%)	1/45 (2%)	7/75 (9%)	4/60 (7%)
Effective rates	0/60 (0%)	1/45 (2%)	7/70 (10%)	4/46 (9%)
Terminal rates	0/59 (0%)	1/39 (3%)	4/32 (13%)	1/10 (10%)
Day of first observation		421 (T)	310	355
Cochran-Armitage test	P=0.021			
Fisher exact test		P=0.429	P=0.011	P=0.033

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the first occurrence of this tumor type in any of the groups
[b] Based on effective rates
[c] No tumors in dosed group or control group; statistical test not performed
[d] 2-year historical incidence for untreated control groups at study laboratory (mean): 0/96 (0%); historical incidence for untreated controls in NTP studies (mean ± SD): 2/1,541 (0.1% ± 0.4%)
[e] 2-year historical incidence for untreated control groups at study laboratory (mean): 0/88 (0%); historical incidence for untreated controls in NTP studies (mean ± SD): 0/1,601 (0% ± 0%)

TABLE 19
Mammary Gland Tumors in Female F344/N Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Adenocarcinoma[a]				
Overall rates	0/60 (0%)	1/45 (2%)	3/75 (4%)	6/60 (10%)
Effective rates[b]	0/60 (0%)	1/45 (2%)	3/71 (4%)	6/51 (12%)
Terminal rates	0/59 (0%)	1/39 (3%)	1/32 (3%)	3/10 (30%)
Day of first observation		421 (T)	363	285
Cochran-Armitage test[c]	P=0.002			
Fisher exact test[c]		P=0.429	P=0.156	P=0.008

(T)Terminal sacrifice
[a] 2-year historical incidence for untreated control groups at study laboratory (mean): 2/100 (2%); historical incidence in NTP studies (mean ± SD): 44/1,643 (2.7% ± 2.2%)
[b] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the first occurrence of this tumor type in any of the groups
[c] Based on effective rates

Mammary Gland: Mammary gland adenocarcinomas, uncommon neoplasms of the female F344/N rat, occurred with a dose-related increased incidence (Table 19). The increase in the high-dose group was significant as compared with controls and was above the highest overall historical incidence for untreated female F344/N rats from 2-year NTP studies [44/1643 (2.7%), range 0-8%]. There was no increase in the incidence of fibroadenomas (control, 2/60; low dose, 1/45; mid dose, 4/75; high dose 0/60).

Lung: Alveolar/bronchiolar adenomas occurred with a slightly increased incidence in treated males relative to untreated controls (Table 20). The incidence of focal or multifocal hyperplasia of alveolar epithelium was markedly increased in treated rats of each sex, while the incidence of histiocytic cellular infiltration of the lung was markedly increased in females (control, 12/60, 20%; low dose, 23/45, 51%; mid dose, 36/74, 49%; high dose, 29/60, 48%). The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was significantly increased in male rats in the mid- and high-dose groups. The lower incidence of alveolar epithelial hyperplasia in high-dose males and females relative to that in mid-dose rats may have been due to the markedly decreased survival of the high-dose groups.

TABLE 20
Lung Tumors in F344/N Rats in the 14-Month Drinking Water Studies of 3,3'-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Male				
Alveolar Epithelial Hyperplasia				
Overall rates	5/60 (8%)	14/45 (31%)	31/75 (41%)	17/60 (28%)
Alveolar/Bronchiolar Adenoma				
Overall rates	1/60 (2%)	0/45 (0%)	7/75 (9%)	6/60 (10%)
Effective rates[a]	1/60 (2%)	0/45 (0%)	7/73 (10%)	6/57 (11%)
Terminal rates	1/60 (2%)	0/41 (0%)	6/50 (12%)	0/0 (0%)
Day of first observation	419 (T)		406	226
Cochran-Armitage test[b]	P=0.012			
Fisher exact test[b]		P=0.571N	P=0.057	P=0.049
Alveolar/Bronchiolar Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	1/75 (1%)	0/60 (0%)
Alveolar/Bronchiolar Adenoma or Carcinoma[c]				
Overall rates	1/60 (2%)	0/45 (0%)	8/75 (11%)	6/60 (10%)
Effective rates	1/60 (2%)	0/45 (0%)	8/73 (11%)	6/57 (11%)
Terminal rates	1/60 (2%)	0/41 (0%)	7/50 (14%)	0/0 (0%)
Day of first observation	419 (T)		406	226
Cochran-Armitage test	P=0.013			
Fisher exact test		P=0.571N	P=0.033	P=0.049
Female				
Alveolar Epithelial Hyperplasia				
Overall rates	1/60 (2%)	11/45 (24%)	30/73 (41%)	13/60 (22%)
Alveolar/Bronchiolar Adenoma				
Overall rates	1/60 (2%)	1/45 (2%)	3/74 (4%)	3/60 (5%)
Effective rates	1/60 (2%)	1/45 (2%)	3/63 (5%)	3/41 (7%)
Terminal rates	1/59 (2%)	1/39 (3%)	2/32 (6%)	0/10 (0%)
Day of first observation	421 (T)	421 (T)	338	370
Cochran-Armitage test	P=0.094			
Fisher exact test		P=0.676	P=0.328	P=0.181
Alveolar/Bronchiolar Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	0/74 (0%)	1/60 (2%)
Alveolar/Bronchiolar Adenoma or Carcinoma[d]				
Overall rates	1/60 (2%)	1/45 (2%)	3/74 (4%)	4/60 (7%)
Effective rates	1/60 (2%)	1/45 (2%)	3/63 (5%)	4/41 (10%)
Terminal rates	1/59 (2%)	1/39 (3%)	2/32 (6%)	0/10 (0%)
Day of first observation	421 (T)	421 (T)	338	370
Cochran-Armitage test	P=0.033			
Fisher exact test		P=0.676	P=0.328	P=0.086

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the first occurrence of this tumor type in any of the groups
[b] Based on effective rates
[c] 2-year historical incidence for untreated control groups at study laboratory (mean): 1/100 (1%); historical incidence for untreated control groups in NTP studies (mean ± SD): 44/1593 (2.8% ± 2.3%)
[d] 2-year historical incidence for untreated control groups at study laboratory (mean): 1/100 (1%); historical incidence for untreated control groups in NTP studies (mean ± SD): 25/1639 (1.5% ± 1.5%)

TABLE 21
Mesotheliomas in Male F344/N Rats in the 14-Month Drinking Water Study of 3,3'-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
All Organs: Mesothelioma (Benign, Malignant, and NOS)[a]				
Overall rates	0/60 (0%)	0/45 (0%)	3/75 (4%)	4/60 (7%)
Effective rates[b]	0/60 (0%)	0/45 (0%)	3/67 (4%)	4/38 (11%)
Terminal rates	0/60 (0%)	0/41 (0%)	3/50 (6%)	0/0
Day of first observation			419 (T)	308
Life table tests	P<0.001	–[d]	P=0.092	P<0.001
Cochran-Armitage test[c]	P=0.003			
Fisher exact test[c]		–[d]	P=0.144	P=0.020

(T)Terminal sacrifice
[a] 2-year historical incidence for untreated control groups at study laboratory (mean): 3/100 (3%); historical incidence for untreated control groups in NTP studies (mean ± SD): 47/1596 (2.9% ± 2.6%)
[b] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the first occurrence of this tumor type in any of the dose groups
[c] No tumors in dosed group or control group; statistical test not performed
[d] Based on effective rates

Mesothelium: Malignant mesotheliomas of the testis and/or epididymis occurred in a few mid- and high-dose males (Table 21). The incidence of mesotheliomas showed a positive trend, and the incidence in high-dose males was significantly increased.

Brain: Small numbers of rare malignant neoplasms of glial cell origin (astrocytoma and glioma) or meningeal origin (malignant meningioma and meningeal sarcoma) occurred in treated male and female rats. Gliomas are poorly differentiated glial cell neoplasms often consisting of a mixture of cells with different morphologies; astrocytomas are better differentiated glial cell neoplasms in which neoplastic cells resemble astrocytes. Malignant gliomas occurred in one mid-dose and one high-dose male and in one mid-dose and one high-dose female. Malignant astrocytomas occurred in one low-dose and one mid-dose female. Malignant meningioma occurred in one low-dose female and one high-dose male; a single meningeal sarcoma, a presumably less-differentiated form of malignant meningioma, occurred in a mid-dose male. Brain neoplasms in control animals are most commonly found at the 2-year terminal sacrifice. The earliest occurring brain neoplasm in this study was the malignant meningioma in the high-dose male that died during week 48. The earliest occurring glial cell neoplasm was the malignant glioma in the high-dose male that died on week 50. The occurrence of these neoplasms solely in treated rats, combined with the early occurrences of these neoplasms, indicates they may have been treatment related.

Adrenal Gland Medulla: Benign pheochromocytomas occurred at a marginally increased incidence in treated male rats (control, 0/60; low dose, 2/45, 4%; mid dose, 1/75, 1%; high dose, 3/59, 5%). The incidence in high-dose males was significantly increased relative to controls. Focal hyperplasia of the adrenal medulla, a lesion generally considered a precursor to pheochromocytoma, was seen in only one mid-dose male. The lack of a dose-related increase in the incidence of pheochromocytomas and the absence of a treatment-related increase in the incidence of hyperplasia in this study indicate the marginal increase in incidence in the high-dose group is not a significant treatment-related effect.

Hematopoietic System: Mononuclear cell leukemia in treated female rats occurred with a slightly increased incidence as compared with controls (control, 1/60; low dose, 3/45; mid dose, 6/75; high dose, 4/60). Mononuclear cell leukemias are generally seen late in life; most cases occur after 18 months of age, and the incidence increases with increasing age. In this study there was substantial early mortality in treated animals due to treatment-related neoplasms in a variety of other tissues, necessitating termination of the study at 14 months. Since the majority of leukemias would not be expected to occur prior to 14 months, the animals in this study were not at risk for a long enough period of time to allow for the full development of leukemias in either control or treated animals. The slightly increased incidences of mononuclear cell leukemia suggest there may have been an earlier onset of leukemia in treated female rats, indicating that the increase in leukemias may have been treatment-related.

Testes: The incidence of interstitial cell adenomas, very common neoplasms in male F344/N rats, was significantly increased in low-dose males as compared with controls (control, 24/60, 40%; low dose, 26/45, 58%; mid dose, 26/75, 35%; high dose 2/60, 3%). However, the incidence of interstitial cell hyperplasia was similar in control and low-dose groups (44/60, 73%; 35/45, 78%; 28/75, 37%; 4/60, 7%). The low incidence in the high-dose group is presumably related to the high early mortality. The increase in the incidence of adenomas in the low-dose group was considered unlikely to be treatment-related because there was no apparent treatment effect on the incidence of interstitial cell hyperplasia, the precursor to adenoma. The lack of a treatment-related increase in hyperplasia is not due to decreased survival since the total number of mid-dose males surviving to study termination (50) was comparable to the number of controls living to termination (60). Consequently, the marginal increase in the incidence of adenomas in the low-dose group is not considered to be treatment related.

Uterus:The incidence of stromal polyps showed a significant negative trend in treated female rats (control, 11/60; low dose, 9/44; mid dose, 3/75; high dose, 1/60). The incidence in mid- and high-dose females was significantly reduced as compared with controls, but was considered secondary to the decreased survival in these groups.

Kidney: Treatment with 3,3′-dimethylbenzidine dihydrochloride was associated with an increased incidence of nephropathy in treated females as compared with controls and a notable increase in the severity of nephropathy in high-dose males and in mid- and high-dose females (Table 22). Nephropathy was characterized by tubule epithelial degeneration and necrosis and accompanied by regenerative tubule cell proliferation that was most marked in males. Minimal karyomegaly, a slight increase in the size of renal tubule cell nuclei, was diagnosed in the kidneys of numerous treated females by the study pathologist. The Pathology Working Group (PWG) reviewed this lesion and believed it to be indicative of regenerative tubule cell proliferation occurring as a part of the nephropathy. Thus, the PWG concluded that karyomegaly in treated females did not represent a distinct treatment-related effect. A single renal tubule adenoma occurred in one high-dose female.

TABLE 22
Incidences and Severity of Nephropathy in Male and Female F344/N Rats in the 14-Month Drinking Water Studies of 3,3'-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Male				
Nephropathy[a]	60/60 (1.7)	43/45 (1.9)	74/75 (2.0)	59/59 (3.4)
Female				
Nephropathy	47/60 (1.3)	44/45 (1.7)	72/74 (2.9)	59/60 (2.9)

[a] Values in parentheses are average severity grades; 1=minimal, 2=mild, 3=moderate, and 4=marked.

Parathyroid Gland: Hyperplasia of the parathyroid gland occurred in 11/58 high-dose males, 3/58 high-dose females, and 5/70 mid-dose females; none was seen in any other group. These lesions are presumed to be secondary to the increased severity of nephropathy in treated rats.

Heart: Minimal to mild cardiomyopathy occurred with increased incidence in treated males (control, 17/60; low dose, 19/45; mid dose, 35/75; high dose 31/59).

Glandular Stomach: Cystic degeneration of the mucosa of the glandular stomach, a common change in aging F344/N rats, consists of atrophy of glandular epithelium and dilatation of the glandular lumens. The incidence of this lesion was increased in treated females (control, 1/60; low dose, 3/45; mid dose, 6/74; high dose, 11/60), but not in males (22/60; 0/45; 4/75; 15/59).

Adrenal Gland Cortex: Angiectasis, characterized by mild dilatation of cortical sinusoids, was seen in treated females (0/60; 0/45; 8/74; 8/60). Cytoplasmic vacuolation of adrenocortical cells occurred with increased frequency in treated males (0/60; 0/44; 5/75; 5/59) and females (2/60; 0/45; 10/74; 6/60).

Spleen: The incidence of hematopoietic cell proliferation was increased in treated males (control, 0/60; low dose, 1/45; mid dose, 22/75; high dose, 11/55) and females (0/60; 6/45; 20/74; 14/60). The increased incidence was considered to be secondary to inflammation associated with neoplasia.

Bone Marrow: Hyperplasia or atrophy of the hematopoietic cell elements of the bone marrow occurred in a few treated males (hyperplasia: mid dose, 1/75; high dose, 3/60; atrophy: high dose, 8/60) and females (hyperplasia: high dose, 7/59; atrophy: mid dose, 1/73; high dose, 4/59). These lesions were not observed in controls.

Mandibular Lymph Node: Plasma cell hyperplasia, characterized by increased numbers of plasma cells within the medullary areas, occurred with increased incidence in treated males (control, 2/60; low dose, 5/45; mid dose, 18/74; high dose, 16/60) and females(1/60; 5/43; 20/72; 18/60). This may have been secondary to the inflammatory response associated with Zymbal's gland neoplasms.

Nose: Suppurative inflammation occurred with increased incidence in mid- and high-dose females (mid-dose, 6/75; high-dose, 5/60).

Seminal Vesicle: The incidence of atrophy was increased in treated male rats (control, 0/60; low dose, 1/44; mid dose, 8/75; high dose, 5/58) and was probably secondary to debilitation in treated males.

GENETIC TOXICITY

3,3′-Dimethylbenzidine dihydrochloride produced positive responses at low doses in several tests for genetic toxicity. 3,3′-Dimethylbenzidine dihydrochloride was tested with a preincubation protocol for induction of gene mutations in *Salmonella typhimurium* strains TA100, TA1535, TA97, and TA98 in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. Mutagenic activity was observed only in strain TA98 in the presence of S9 (Zeiger et al., 1988; Table C1). In cytogenetic tests with Chinese hamster ovary cells, 3,3′-dimethylbenzidine dihydrochloride induced sister-chromatid exchanges (SCE) (Table C2) and chromosomal aberrations (Table C3) in the absence of S9. Neither endpoint was elevated in trials conducted with S9 from Aroclor 1254-induced male Sprague-Dawley rat liver. In the SCE tests, positive responses were recorded in each of two trials without S9. In the chromosomal aberration assay, the first of two trials without S9 was negative, but in the second trial three intermediate dose levels produced significant increases in aberrations. 3,3′-Dimethylbenzidine dihydrochloride induced sex-linked recessive lethal mutations in the germ cells of male *Drosophila* when administered either in feed or by injection (Table C4). No induction of reciprocal translocations occurred in *Drosophila* germ cells following exposure of males by feeding (Table C5). Appendix C contains the methods and complete results of all genetic toxicology studies.

DISCUSSION AND CONCLUSIONS

Consumption of drinking water containing 3,3′-dimethylbenzidine dihydrochloride by rats led to highly significant increased incidences of neoplasms at a variety of sites and mild toxicity in several organs. In low-, mid-, and high-dose males, 13%, 64%, and 83%, respectively, were observed to have malignant neoplasms, and many animals in the mid- and high-dose groups had multiple malignant neoplasms. Similarly, malignant neoplasms were found in 31%, 65%, and 93% of low-, mid-, and high-dose females, with many animals in all dose groups having malignant neoplasms at multiple sites. Only 2% of male or female control rats had malignant neoplasms.

The principal sites and organs with neoplasms included the skin, Zymbal's gland, preputial and clitoral glands, liver, oral mucosa, and small and large intestine. In both sexes, the incidence of these neoplasms was dose related, and the tumor latency generally decreased with increasing dose. The occurrence of neoplasms at most of these sites in the F344/N rat is uncommon and often associated with exposure to genotoxic carcinogens. The short latency and multiple sites of these neoplasms are characteristic of genotoxic carcinogens such as the benzidine dyes (NCI, 1978a), 3,3′-dimethoxybenzidine (NTP, 1990a), benzene (NTP, 1986), 1,3-butadiene (NTP, 1984), and glycidol (NTP, 1990b).

14-DAY AND 13-WEEK STUDIES

In the 14-day and 13-week studies, male and female rats were exposed to 3,3′-dimethylbenzidine dihydrochloride in drinking water at concentrations ranging from 300 to 7,500 ppm. In the 14-day studies, all five males and one female receiving 7,500 ppm and 1/5 males receiving 5,000 ppm 3,3′-dimethylbenzidine dihydrochloride died. In the 13-week studies, all animals receiving 4,000 ppm and 4/10 males and 3/10 females receiving 2,000 ppm 3,3′-dimethylbenzidine dihydrochloride died. Mean necropsy body weights showed a dose-related decrease. Histopathologic evidence of hepatic (necrosis and pigment within the sinusoidal lining cells) and renal damage (increased severity of nephropathy) was seen in exposed rats. In addition,lymphocytic atrophy was observed in the thymus, mandibular and mesenteric lymph nodes, and spleen of treated animals, and atrophy of the bone marrow was seen in rats receiving 2,000 and 4,000 ppm. Water consumption was decreased with increasing chemical concentration.

Small dose-related decreases in hematocrit values indicated a slight anemia in males and females; however, the lack of a concomitant decrease in hemoglobin levels suggested that these decreases were a result of hemolysis. The slight increase in serum sorbitol dehydrogenase (SDH) activity in treated rats was indicative of mild liver damage.

Based on the decreased survival, reductions in dosed water consumption and body weight gain, and chemical-induced hepatocellular and renal lesions observed in the 13-week studies, the 9- and 14-month studies were conducted in male and female rats by administering 0, 30, 70, or 150 ppm 3,3′-dimethylbenzidine dihydrochloride in drinking water.

9-MONTH STUDIES

Carcinomas of the preputial and clitoral glands, Zymbal's gland, liver, skin, lung, oral cavity, and small intestine were observed in high-dose animals after exposure to 3,3′-dimethylbenzidine dihydrochloride for only 9 months. Basophilic foci and neoplastic nodules in the liver and hyperplasias of the Zymbal's gland and lung were also detected in exposed rats. These lesions were not detected in control rats. The short latency of these lesions is unusual and indicative of the carcinogenic potency of 3,3′-dimethylbenzidine dihydrochloride.

Hematologic effects indicated a mild anemia in treated male and female rats. Serum SDH levels were increased about tenfold in females and two-fold in males and were indicative of liver injury. A decrease in serum thyroxin (T_4) with an increase in thyroid stimulating hormone (TSH) is indicative of mild hypothyroidism in treated male and female rats.

14-MONTH STUDIES

3,3′-Dimethylbenzidine dihydrochloride studies were terminated at month 14 because of reduced survival in the dosed groups. All high-dose males had been found dead or killed moribund by week 55, and only about 25% of the high-dose females survived to week 56. Survival was influenced, in part, by an aggressive sacrifice program that, for humane reasons and to preclude autolysis, called for removal of animals with large ulcerated neoplasms. Mean body weights of the high-dose male and female groups were approximately 85% of those of corresponding control groups by weeks 28 and 32, respectively, and 70% and 75% by study termination, respectively. By week 44, the mean body weight of the mid-dose female group was 85% of that of the control group.

Clinical Findings

The most important clinical finding in the 14-month studies was the appearance of tissue masses on the head, over the back, and in the genital area. These masses, for the most part, represented the development of Zymbal's gland tumors, epithelial skin tumors, and preputial/clitoral gland tumors, respectively. Tissue masses at these three sites had a relatively short latency, with tissue masses on the head first appearing after 24 weeks of chemical exposure.

Nonneoplastic Lesions

3,3′-Dimethylbenzidine dihydrochloride exposure was associated with increased incidences of several nonneoplastic lesions. Cystic degeneration, a common degenerative change in the rat liver, was observed in the liver of exposed rats and appeared to be treatment related. Increased hematopoiesis in the liver was likely secondary to inflammation associated with neoplasms. A treatment-related increase in the incidence of nephropathy was observed in female rats, and an increase in the severity of nephropathy was observed in high-dose males and mid- and high-dose females. Although treatment related, the increase in alveolar macrophages (histiocytic cellular infiltration) observed in the lung of treated females was probably a nonspecific reaction and possibly a sequela to hyperpnea related to anemia or stress.

Neoplastic Lesions

Of the male rats exposed to 150 ppm 3,3′-dimethylbenzidine dihydrochloride in drinking water, 50% were found to have skin basal cell tumors, and 45% had squamous cell skin neoplasms. These neoplasms were not observed in untreated controls. Epithelial skin neoplasms were composed principally of basal or squamous cells. In treated males, basal cell tumors occurred as early as week 40 (observed at necropsy), and squamous cell tumors as early as week 30. Although the incidence of epithelial skin tumors in treated females was not as remarkable as that in males, the incidence of these neoplasms was significantly increased by 3,3′-dimethylbenzidine dihydrochloride treatment. Skin neoplasms detected in treated females were of the same morphological type as those observed in males and were a result of 3,3′-dimethylbenzidine dihydrochloride exposure.

Skin neoplasms could have been caused by systemic exposure to reactive 3,3′-dimethylbenzidine metabolites or by direct exposure to 3,3′-dimethylbenzidine in the drinking water. Because 3,3′-dimethylbenzidine dihydrochloride was administered in drinking water, exposure of skin during grooming was likely. Skin neoplasms may have resulted from direct exposure of the skin to the compound or to its metabolites in saliva, or from metabolism by the skin of 3,3′-dimethylbenzidine dihydrochloride to a reactive intermediate. No reports on the carcinogenicity of 3,3′-dimethylbenzidine dihydrochloride after dermal administration were found.

There was a highly significant correlation between the consumption of 3,3′-dimethylbenzidine dihydrochloride and the development of Zymbal's gland adenomas and/or carcinomas in treated males and females. With the exception of an adenoma in one control male, Zymbal's gland neoplasms were not observed in control groups. Carcinomas and adenomas were observed at necropsy in treated males as early as weeks 30 and 36, respectively, and in treated females, as early as weeks 26 and 36, respectively. Neoplasms infrequently develop spontaneously at this site (1% of untreated historical control rats) and usually only late in life (Solleveld *et al.*, 1984).

Exposure to 3,3′-dimethylbenzidine dihydrochloride had a profound effect on the clitoral gland in treated female rats, giving rise to a high incidence of adenomas and/or carcinomas. The incidence of

these neoplasms in high-dose females was about 8 times higher than those in untreated historical control F344/N rats in 2-year studies. Adenomas were found in treated female animals as early as week 33, and carcinomas as early as week 36. Potential precursor lesions (hyperplasia) occurred in small numbers in treated animals, possibly because most such lesions had already progressed to neoplasms. An increased incidence of neoplasms was also observed in the preputial gland of treated males. Although not as marked as the incidence of clitoral gland tumors in treated females, the incidence of preputial gland neoplasms in treated males was about three times higher than that of laboratory historical controls. Preputial gland adenomas and carcinomas were confirmed histologically as early as weeks 40 and 44, respectively. Adenomas of the preputial gland were observed in two control rats at 60 weeks (historical incidence in untreated controls 0% at study laboratory, 4.3% in NTP studies).

Intake of 3,3′-dimethylbenzidine dihydrochloride was associated with an increased incidence of hepatocellular neoplasms, principally neoplastic nodules (hepatocellular adenoma), in treated male and female rats. The incidence of hepatocellular neoplasms in treated female rats was considerably lower than in males. The incidence of neoplastic nodules or carcinomas (combined) was significantly ($P \leq 0.05$) increased for mid- (47%) and high-dose (55%) males and mid- (9%) and high-dose (7%) females; no hepatocellular neoplasms occurred in the untreated or low-dose groups of either sex. It was therefore concluded that 3,3′-dimethylbenzidine dihydrochloride treatment was responsible for these neoplasms in male and female rats. 3,3′-Dimethylbenzidine dihydrochloride was also associated with an increase in the incidence of basophilic, eosinophilic and mixed cell foci in male and female rats, which, if these foci are considered precursor lesions, strengthens the conclusion that 3,3′-dimethylbenzidine dihydrochloride is a hepatocarcinogen. The chemical also caused mild hepatotoxicity.

Squamous cell neoplasms occurring in the oral cavity (tongue and palate) of treated females were strongly associated with exposure to 3,3′-dimethylbenzidine dihydrochloride. Significant numbers of squamous cell tumors of the oral cavity were also detected in treated male rats although at a lower incidence. Neoplasms of the oral cavity could have been caused by direct exposure to 3,3′-dimethylbenzidine dihydrochloride in the drinking water or by systemic exposure to reactive 3,3′-dimethylbenzidine dihydrochloride metabolites. Taken collectively, the observed number of squamous cell papillomas and carcinomas of the oral cavity represents a comparatively large increase in the incidence of relatively rare tumors (historical incidence of 4/1,643, 0.2%, in untreated female F344/N rats in 2-year studies). It was concluded that these tumors were caused by 3,3′-dimethylbenzidine dihydrochloride treatment in male and female rats.

3,3′-Dimethylbenzidine dihydrochloride exposure led to development of uncommon epithelial neoplasms of the small and large intestine in male and female rats. Chemically induced neoplasms of the intestine are uncommon in rats. Of 370 chemicals studied by the NCI/NTP, only seven were associated with adenocarcinomas, adenomatous polyps, or carcinomas of the intestine in the rat: 3,3′-dimethoxybenzidine (NTP, 1990a), tribromomethane (NTP, 1989), bromodichloromethane (NTP, 1987), Captan (NCI, 1977), phenazopyridine hydrochloride (NCI, 1978b), chrysotile asbestos (NTP, 1985), and glycidol (NTP, 1990b).

The neoplasms in the current study were principally cystic mucinous adenocarcinomas of the small intestine and adenomatous polyps of the large intestine. Adenocarcinomas of the small intestine first occurred after 30 and 36 weeks of treatment in males and females, respectively, and colonic polyps were first observed at necropsy at week 44 in males and females. Adenocarcinomas were also observed in the large intestine of seven high-dose males and one mid-dose and one high-dose female. Although the increase in incidence of these tumors in females was not as marked as in males, these tumors were considered due to 3,3′-dimethylbenzidine dihydrochloride exposure since no adenocarcinomas or adenomatous polyps have been observed in 1,601 untreated historical control female F344/N rats in 2-year NTP studies.

3,3′-Dimethylbenzidine dihydrochloride consumption led to a dose-related increase in the incidence and a shortened latency of adenocarcinomas of the mammary gland of female rats. The incidence of adenocarcinomas was statistically significant only in the high-dose group; no adenocarcinomas were observed in untreated control rats. This neoplasm was first observed at necropsy in high-dose females at week 41, in mid-dose females at week 52, and in

low-dose females at week 60. Based upon the dose-related increase in incidence of adenocarcinomas and decrease in time-to-tumor, it was concluded that mammary gland neoplasms were a result of 3,3′-dimethylbenzidine dihydrochloride treatment.

The incidence of alveolar/bronchiolar neoplasms of the lung was significantly increased in mid- and high-dose male rats. A dose-related increase in the incidence of these lung tumors occurred in female rats and was significant in the high-dose group. Hyperplasia of the alveolar epithelium occurred in up to 41% of treated male and female rats. Because alveolar/bronchiolar tumors are uncommon in the F344 rat (2.8% or 1.5% in untreated male or female control rats in NTP 2-year studies) and because of the high treatment-related incidences of hyperplasia, alveolar/bronchiolar tumors were considered directly related to 3,3′-dimethylbenzidine dihydrochloride treatment.

A few uncommon malignant neoplasms of glial cell or meningeal origin occurred in the brains of treated male and female rats, but not in controls. The first neoplasms were observed at week 50 and 55 in high-dose males and females, respectively, and at week 60 in mid-dose males and females. The incidence of these tumors was only marginally increased, and was not dose related. However, in view of the reduced survival of treated rats and low spontaneous occurrence of these tumors (historical incidence <1.0% for any of these tumors in NTP 2-year studies), these neoplasms may have been related to 3,3′-dimethylbenzidine dihydrochloride exposure.

An increased incidence of mesotheliomas in male rats was associated with 3,3′-dimethylbenzidine dihydrochloride treatment in the mid-dose (3/75, 4%) and high-dose (4/60, 7%) groups. Mesotheliomas were not detected in untreated male control or low-dose rats. The laboratory control incidence (2/100) was similar to the overall historical incidence of malignant mesotheliomas in male F344/N rats (0.7%, 11/1,596) in 2-year NTP studies. Although the increased incidence of mesotheliomas in the mid- and high-dose rats was not as marked as that of other neoplasms, it is possible that the incidence would have been higher had the animals in these groups survived longer. In consideration of the decreased survival and moderately increased incidence of mesotheliomas in these animals, it was concluded that these tumors were a result of 3,3′-dimethylbenzidine dihydrochloride treatment.

Survival of 3,3′-dimethylbenzidine dihydrochloride-exposed rats was reduced during the 14-month studies, primarily because of the number of moribund sacrifices associated with the presence of grossly visible neoplasms of the skin, Zymbal's gland, and preputial and clitoral glands in male and female rats. Tumors of these tissues first appeared in males after treatment for 30 weeks (Zymbal's gland and skin) and in females after 26 weeks (Zymbal's gland). Early mortality from these tumors may have reduced the number of male and female rats at risk for development of tumors at other sites.

For these later developing or less rapidly lethal tumors, expression of tumor incidence by the standard convention (the number of animals with tumors at a site divided by the total number of animals in which this site was examined) may underestimate the tumor incidence which would have been observed in the absence of early deaths. Therefore, tumor incidence ratios were expressed in terms of the "effective" number of animals actually at risk, i.e., the number of animals bearing a tumor at a particular site by the number of animals alive in each group at the time the first tumor was observed at that site in any of the four (control, low, mid, or high dose) groups. These derived incidences were analyzed statistically with the Cochran-Armitage trend test and the Fisher exact test.

ONCOGENE ACTIVATION

Neoplasms obtained from control rats and rats treated with 3,3′-dimethylbenzidine dihydrochloride or C.I. Acid Red 114 (a 3,3′-dimethylbenzidine-derived dye) were assayed for the presence of activated proto-oncogenes by the NIH 3T3 DNA transfection assay (Anderson *et al.*, 1987; Reynolds *et al.*, 1990). Oncogenes detectable by DNA transfection analysis were present in 13/14 skin or clitoral gland neoplasms induced by 3,3′-dimethylbenzidine dihydrochloride or C.I. Acid Red 114. DNA from both benign and malignant neoplasms was capable of inducing morphologically transformed foci in NIH 3T3 mouse fibroblast cultures. Oncogenes were not detectable in one fibrosarcoma and three mammary fibroadenomas in treated rats.

Fourteen of the 18 chemically induced tumors were of epidermal origin, and activated *ras* oncogenes

were detected at a high frequency in these tumors (12/14). Neoplasms of the clitoral glands had a high frequency of activated *ras* oncogenes (4/4).

It is difficult to compare oncogene activation in spontaneously occurring tumors with that in chemically induced tumors because of the substantial difference in the tumor types obtained in the two groups. Only 55% (21/38) of the spontaneously occurring tumors were of epithelial cell origin. However, in comparing the tumors of epithelial cell origin, there was a 15-fold higher incidence of *ras* gene activation in the chemically induced tumors (13/18) than in the spontaneous tumors (1/21).

It is possible that chemically induced neoplasms were derived from a common epidermal progenitor stem-cell population that was susceptible to electrophilic attack by activated metabolites of 3,3′-dimethylbenzidine dihydrochloride or C.I. Acid Red 114. A relatively high percentage (62%) of the chemically induced rat neoplasms contained activated alleles of either H-*ras* or N-*ras*. Those neoplasms with activated H-*ras* contained point mutations in codons 12, 13, or 61. The much higher incidence of H-*ras* gene activation and apparent mutational specificity at codons 13 and 61 of H-*ras* with 3,3′-dimethylbenzidine dihydrochloride exposure suggest that the increased tumor incidence observed in treated rats is directly related to the genotoxic effect of this chemical.

Related Aromatic Amines

Benzidine and related aromatic amines produce tumors 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, however, the liver is the major target organ (Bonser *et al.*, 1956; Vesselinovitch *et al.*, 1975; Littlefield *et al.*, 1983). In rats 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine, benzidine, and other aminobiphenyls cause tumors in the Zymbal's gland, mammary gland, skin, intestine, and liver. 3,3′-Dimethylbenzidine caused tumors in the lung of both rats and mice. Although the mechanism is not entirely clear, these differences in species and target organ specificity appear to be related to differences in metabolism.

A number of aromatic amines cause tumors of the Zymbal's gland; however, the basis for this organ specificity is poorly understood. The Zymbal's gland has been reported to be deficient in sulfotransferase activity (Irving *et al.*, 1971) and transacylase activity (Bartsch *et al.*, 1973), but 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 upon the ability of the species to *N*-hydroxylate the amine substituent. *N*-hydroxylation appears to be a necessary but insufficient step in the metabolic activation of aromatic amines, and subsequent formation of an ester is required, resulting in an active electrophilic agent (Miller and Miller, 1977). Formation of different esters by different species may result in variations in organ specificity (Cohen, 1983).

Of 370 chemicals evaluated for carcinogenicity in rats and mice by the NCI/NTP, only 15 were associated with Zymbal's gland neoplasms in rats. Ten of these 15 are aryl nitrogen derivatives (nitro, amino, or isocyanate) that were mutagenic for *Salmonella typhimurium* and produced neoplasms in both rats and mice. In a survey of 222 chemicals evaluated for carcinogenicity in rats and mice by the NCI/NTP (Ashby and Tennant, 1988), only nine chemicals were associated with Zymbal's gland tumors in the rat. Eight of these chemicals were aryl nitrogen derivatives, were mutagenic for *S. typhimurium*, and produced tumors in both rats and mice. Only six of the 222 chemicals surveyed were associated with skin tumors following systemic administration. Of these six chemicals, five were aryl nitrogen derivatives, and five were among the group of nine chemicals which caused Zymbal's gland tumors. Although not included in this survey, 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine, benzidine, and several other aromatic amines also belong to this unique group of genotoxic carcinogens that cause Zymbal's gland and/or skin tumors in rodents (Table 23).

3,3′-Dimethoxybenzidine, a related benzidine congener, was studied simultaneously with 3,3′-dimethylbenzidine using the same study design (NTP, 1990a). The 3,3′-dimethoxybenzidine study was also terminated early (21 months) because of poor animal survival due to neoplasia. Both 3,3′-dimethoxybenzidine and 3,3′-dimethylbenzidine are potent carcinogens affecting principally the skin, Zymbal's gland, clitoral and preputial glands, liver, oral cavity,

TABLE 23
Structural Analogs of 3,3′-Dimethylbenzidine That Are Mutagenic Carcinogens for the Zymbal's Gland and Skin in Rats

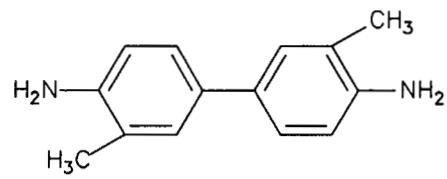
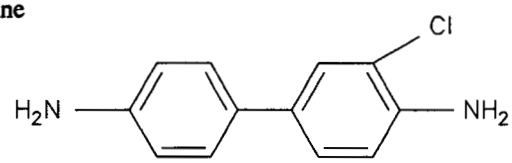
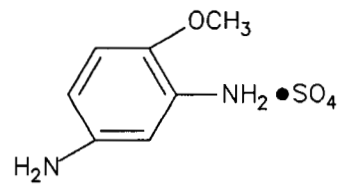
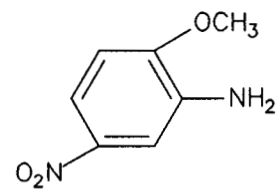
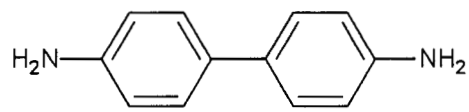
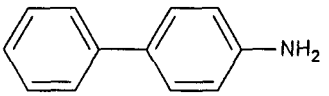
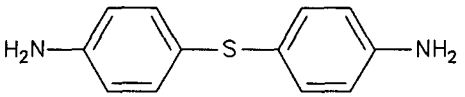
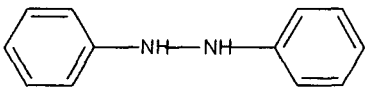
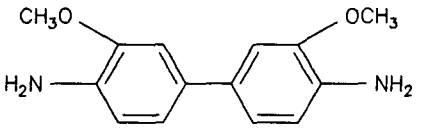
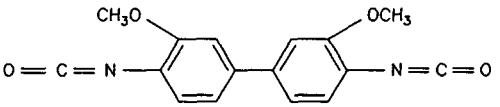
Aromatic Amine	Structure	*Salmonella typhimurium* Assay	Zymbal's Gland	Skin	References
3,3′-Dimethylbenzidine		+	+	+	Pliss, 1965; Current studies
3,3′-Dichlorobenzidine		+	+	+	IARC, 1987; Lazear and Louie, 1977
2,4-Diaminoanisole sulfate		+	+	+	NCI, 1978e
5-Nitro-*o*-anisidine		+	+	+	NCI, 1978f
Benzidine		+	+	-	IARC, 1987

TABLE 23
Structural Analogs of 3,3′-Dimethylbenzidine That Are Mutagenic Carcinogens for the Zymbal's Gland and Skin in Rats (continued)

Aromatic Amine	Structure	*Salmonella typhimurium* Assay	Zymbal's Gland	Skin	References
4-Aminobiphenyl		+	+	–	IARC, 1987
4,4′-Thiodianiline		+	+	+	NCI, 1978c
Hydrazobenzene		+	+	–	NCI, 1978d
3,3′-Dimethoxybenzidine		+	+	+	NTP, 1990a
3,3′-Dimethoxybenzidine diisocyanate		+	+	+	NCI, 1979b

and intestine in the F344/N rat. In addition, both benzidine congeners caused increased incidences in neoplasms in the mesothelium, mammary gland, and brain. Although the increase in the incidence of neoplasms in these organs was less remarkable, the fact that both related chemicals caused lesions at these sites further supports its significance.

3,3′-Dimethylbenzidine caused alveolar/bronchiolar tumors in F344/N rats, whereas 3,3′-dimethoxybenzidine did not. In studies conducted at the NCTR, 3,3′-dimethoxybenzidine was negative for these tumors in BALB/c mice, and 3,3′-dimethylbenzidine caused a low incidence of lung tumors. The reasons for these species and target site differences are not clear.

CONCLUSION

Under the conditions of these 14-month drinking water studies, there was *clear evidence of carcinogenic activity** of 3,3′-dimethylbenzidine dihydrochloride for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity, small and large intestine, mesothelium, and lung. Increased incidences of neoplasms of the brain may have been related to chemical administration. There was *clear evidence of carcinogenic activity* for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity, small and large intestine, mammary gland, and lung. Increased incidences of neoplasms of the brain and mononuclear cell leukemia may have been related to chemical administration.

* 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 appears on page 11.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 14-MONTH DRINKING WATER STUDY OF 3,3′-DIMETHYLBENZIDINE DIHYDROCHLORIDE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
Scheduled sacrifice	10			10
Early deaths				
Moribund		2	19	45
Died last week of study		2	5	15
Accident			1	
Survivors				
Terminal sacrifice	60	41	49	
Dead			1	
Animals examined microscopically	60	45	75	60
Alimentary System				
Intestine large	(60)	(45)	(75)	(60)
Adenocarcinoma				1 (2%)
Intestine large, cecum	(60)	(45)	(75)	(58)
Lymphoma malignant histiocytic			1 (1%)	
Polyp adenomatous			1 (1%)	1 (2%)
Intestine large, colon	(60)	(45)	(75)	(57)
Adenocarcinoma				4 (7%)
Adenocarcinoma, cystic, mucinous				1 (2%)
Polyp adenomatous			5 (7%)	3 (5%)
Polyp adenomatous, multiple				2 (4%)
Intestine large, rectum	(60)	(44)	(75)	(60)
Adenocarcinoma				1 (2%)
Polyp adenomatous				3 (5%)
Intestine small, duodenum	(60)	(44)	(75)	(56)
Adenocarcinoma, cystic, mucinous			2 (3%)	
Intestine small, ileum	(60)	(44)	(75)	(54)
Adenocarcinoma, multiple				1 (2%)
Polyp adenomatous			1 (1%)	1 (2%)
Intestine small, jejunum	(60)	(44)	(75)	(55)
Adenocarcinoma, cystic, mucinous			1 (1%)	6 (11%)
Adenocarcinoma, cystic, mucinous, multiple				1 (2%)
Liver	(60)	(45)	(75)	(60)
Cholangiocarcinoma				1 (2%)
Hepatoblastoma				1 (2%)
Hepatocellular carcinoma			11 (15%)	10 (17%)
Hepatocellular carcinoma, multiple			1 (1%)	2 (3%)
Leukemia mononuclear		1 (2%)		1 (2%)
Lymphoma malignant histiocytic			1 (1%)	
Lymphoma malignant undifferentiated cell type			1 (1%)	
Neoplastic nodule			15 (20%)	15 (25%)
Neoplastic nodule, multiple			14 (19%)	11 (18%)
Pancreas	(60)	(44)	(75)	(57)
Leukemia mononuclear				1 (2%)
Lymphoma malignant histiocytic			1 (1%)	
Pharynx			(3)	(3)
Papilloma squamous			2 (67%)	2 (67%)
Squamous cell carcinoma			1 (33%)	1 (33%)
Stomach, glandular	(60)	(45)	(75)	(59)
Leukemia mononuclear		1 (2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Alimentary System (continued)				
Tongue			(1)	(3)
Papilloma squamous			1 (100%)	
Squamous cell carcinoma				2 (67%)
Cardiovascular System				
Heart	(60)	(45)	(75)	(59)
Leukemia mononuclear		1 (2%)		1 (2%)
Lymphoma malignant histiocytic			1 (1%)	
Endocrine System				
Adrenal gland, cortex	(60)	(44)	(75)	(59)
Leukemia mononuclear		1 (2%)		1 (2%)
Adrenal gland, medulla	(60)	(45)	(75)	(59)
Leukemia mononuclear				1 (2%)
Pheochromocytoma benign		2 (4%)	1 (1%)	3 (5%)
Pituitary gland	(60)	(45)	(75)	(60)
Leukemia mononuclear				1 (2%)
Pars distalis, adenoma	1 (2%)	2 (4%)	4 (5%)	1 (2%)
Thyroid gland	(60)	(44)	(75)	(60)
C-cell, adenoma	2 (3%)	1 (2%)	3 (4%)	1 (2%)
Follicular cell, adenoma	1 (2%)	1 (2%)	1 (1%)	1 (2%)
General Body System				
Tissue NOS			(1)	(4)
Sarcoma				1 (25%)
Genital System				
Epididymis	(60)	(45)	(75)	(60)
Leukemia mononuclear		1 (2%)		
Lymphoma malignant histiocytic			1 (1%)	
Mesothelioma malignant				1 (2%)
Mesothelioma malignant, metastatic, testes			2 (3%)	3 (5%)
Preputial gland	(60)	(45)	(75)	(60)
Adenoma	2 (3%)	4 (9%)	4 (5%)	8 (13%)
Carcinoma			2 (3%)	1 (2%)
Testes	(60)	(45)	(75)	(60)
Leukemia mononuclear		1 (2%)		1 (2%)
Lymphoma malignant histiocytic			1 (1%)	
Mesothelioma malignant			3 (4%)	3 (5%)
Bilateral, interstitial cell, adenoma	7 (12%)	9 (20%)	6 (8%)	1 (2%)
Interstitial cell, adenoma	17 (28%)	17 (38%)	20 (27%)	1 (2%)
Hematopoietic System				
Bone marrow	(60)	(44)	(75)	(60)
Leukemia mononuclear		1 (2%)		1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Hematopoietic System (continued)				
Lymph node	(60)	(45)	(75)	(60)
Mediastinal, leukemia mononuclear		1 (2%)		1 (2%)
Mediastinal, lymphoma malignant histiocytic			1 (1%)	
Mediastinal, squamous cell carcinoma, metastatic, skin		1 (2%)		
Pancreatic, leukemia mononuclear				1 (2%)
Lymph node, mandibular	(60)	(45)	(74)	(60)
Leukemia mononuclear		1 (2%)		
Squamous cell carcinoma, metastatic, skin				1 (2%)
Lymph node, mesenteric	(60)	(44)	(75)	(56)
Leukemia mononuclear		1 (2%)		
Lymphoma malignant histiocytic			1 (1%)	
Spleen	(60)	(45)	(75)	(55)
Fibroma				1 (2%)
Leukemia mononuclear		1 (2%)	1 (1%)	1 (2%)
Integumentary System				
Skin	(60)	(44)	(75)	(60)
Basal cell adenoma		7 (16%)	17 (23%)	8 (13%)
Basal cell adenoma, multiple		3 (7%)	35 (47%)	21 (35%)
Basal cell carcinoma		1 (2%)	4 (5%)	2 (3%)
Keratoacanthoma	1 (2%)	1 (2%)	7 (9%)	5 (8%)
Keratoacanthoma, multiple			1 (1%)	
Leukemia mononuclear		1 (2%)		
Papilloma squamous			8 (11%)	13 (22%)
Papilloma squamous, multiple				2 (3%)
Squamous cell carcinoma		1 (2%)	8 (11%)	10 (17%)
Squamous cell carcinoma, multiple		1 (2%)	2 (3%)	3 (5%)
Sebaceous gland, adenoma			6 (8%)	4 (7%)
Sebaceous gland, adenoma, multiple			1 (1%)	1 (2%)
Subcutaneous tissue, fibroma				2 (3%)
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, schwannoma benign			1 (1%)	
Musculoskeletal System				
None				
Nervous System				
Brain	(60)	(45)	(75)	(60)
Carcinoma, metastatic, Zymbal's gland			1 (1%)	
Glioma malignant			1 (1%)	
Glioma malignant, focal, mild				1 (2%)
Leukemia mononuclear		1 (2%)		1 (2%)
Lymphoma malignant histiocytic			1 (1%)	
Meningioma malignant				1 (2%)
Cranial nerve, meninges, carcinoma, metastatic, Zymbal's gland				1 (2%)
Meninges, sarcoma			1 (1%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Respiratory System				
Lung	(60)	(45)	(75)	(60)
Alveolar/bronchiolar adenoma	1 (2%)		6 (8%)	4 (7%)
Alveolar/bronchiolar adenoma, multiple			1 (1%)	2 (3%)
Alveolar/bronchiolar carcinoma			1 (1%)	
Carcinoma, metastatic, Zymbal's gland		1 (2%)	3 (4%)	3 (5%)
Leukemia mononuclear				1 (2%)
Lymphoma malignant histiocytic			1 (1%)	
Squamous cell carcinoma, metastatic, skin		1 (2%)		3 (5%)
Nose	(60)	(45)	(75)	(60)
Leukemia mononuclear		1 (2%)		
Special Senses System				
Ear			(2)	
Squamous cell carcinoma				1 (50%)
Canal, papilloma squamous				1 (50%)
Zymbal's gland	(59)	(45)	(75)	(59)
Adenoma	1 (2%)	1 (2%)	13 (17%)	13 (22%)
Carcinoma		2 (4%)	20 (27%)	22 (37%)
Bilateral, adenoma				3 (5%)
Bilateral, carcinoma			1 (1%)	1 (2%)
Urinary System				
Kidney	(60)	(45)	(75)	(59)
Leukemia mononuclear		1 (2%)		1 (2%)
Lipoma			1 (1%)	
Lymphoma malignant histiocytic			1 (1%)	
Urinary bladder	(60)	(45)	(75)	(59)
Leukemia mononuclear				1 (2%)
Systemic Lesions				
Multiple organs	(60)*	(45)*	(75)*	(60)*
Leukemia mononuclear		1 (2%)	1 (1%)	1 (2%)
Lymphoma malignant histiocytic			1 (1%)	
Lymphoma malignant undifferentiated cell			1 (1%)	
Mesothelioma malignant			3 (4%)	4 (7%)
Tumor Summary				
Total animals with primary neoplasms**	28	38	73	58
Total primary neoplasms	34	54	237	214
Total animals with benign neoplasms	28	34	66	47
Total benign neoplasms	33	48	175	134
Total animals with malignant neoplasms	1	6	48	50
Total malignant neoplasms	1	6	62	80
Total animals with secondary neoplasms***		2	5	11
Total secondary neoplasms		3	6	11

* Number of animals with any tissue examined microscopically
** Primary tumors: all tumors except metastatic tumors
*** Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm

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

+: Tissue examined
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 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm (continued)

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm (continued)

Number of Days on Study	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 6	
Carcass ID Number	1 3 2	1 3 3	1 3 4	1 3 5	1 4 1	1 4 2	1 4 3	1 4 4	1 4 5	1 0 5 3	**Total Tissues/ Tumors**
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	60
Intestine large	+	+	+	+	+	+	+	+	+	+	60
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	60
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	60
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	60
Intestine small	+	+	+	+	+	+	+	+	+	+	60
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	60
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	60
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	60
Liver	+	+	+	+	+	+	+	+	+	+	60
Mesentery											2
Pancreas	+	+	+	+	+	+	+	+	+	+	60
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Stomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	60
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	60
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	60
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	60
Parathyroid gland	M	+	+	+	+	+	+	+	+	+	55
Pituitary gland	+	+	+	+	+	+	+	+	+	+	60
Pars distalis, adenoma											1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	60
C-cell, adenoma											2
Follicular cell, adenoma											1
General Body System											
None											

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm (continued)

Number of Days on Study	419	419	419	419	419	419	419	419	419	419	419	419	419	419	419	420	420	420	420	420	420	420	420	420	420	420
Carcass ID Number	03311	03312	03313	03314	03315	03441	03442	03443	03444	03445	03551	03552	03554	03555	03561	03562	03563	03564	03565	03571	03572	03573	03574	03575	03581	
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma			X																							
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Bilateral, interstitial cell, adenoma			X			X															X					
Interstitial cell, adenoma	X	X		X							X		X					X						X	X	
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Thymus	M	M	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Integumentary System																										
Mammary gland	+	M	+	+	M	+	+	M	+	M	M	+	+	+	M	M	+	+	M	M	M	+	+	+	+	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Keratoacanthoma																										
Subcutaneous tissue, fibrosarcoma																										
Musculoskeletal System																										
None																										
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Alveolar/bronchiolar adenoma																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Special Senses System																										
Eye								+																		
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm (continued)

Number of Days on Study	420	420	420	420	420	420	420	420	420	420	421	421	421	421	421	421	421	421	421	421	421	421	421	421	421
Carcass ID Number	08223	08234	08245	08291	09012	09023	09034	09045	09051	09102	09113	09123	09134	09145	09201	09212	09223	09234	09245	09251	09262	09273	09284	09295	09301
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
adenoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma											X		X							X		X			
Interstitial cell, adenoma					X	X		X		X		X					X				X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	M	+	M	+	+	+	+	+	+	+	M	+	M	+	+	+	+	M	+	+
Integumentary System																									
Mammary gland	+	M	+	+	+	+	M	M	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																			X						
Subcutaneous tissue, fibrosarcoma																			X						
Musculoskeletal System																									
None																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																					X				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								X	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm (continued)

Number of Days on Study	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 6	
Carcass ID Number	1 3 2	1 3 3	1 3 4	1 3 5	1 4 1	1 4 2	1 4 3	1 4 4	1 4 5	0 5 3	**Total Tissues/ Tumors**
Genital System											
Epididymis	+	+	+	+	+	+	+	+	+	+	60
Preputial gland	+	+	+	+	+	+	+	+	+	+	60
Adenoma										X	2
Prostate	+	+	+	+	+	+	+	+	+	+	60
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	60
Testes	+	+	+	+	+	+	+	+	+	+	60
Bilateral, interstitial cell, adenoma											7
Interstitial cell, adenoma								X		X	17
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	60
Lymph node	+	+	+	+	+	+	+	+	+	+	60
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	60
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	60
Spleen	+	+	+	+	+	+	+	+	+	+	60
Thymus	+	I	I	+	+	+	+	+	+	M	47
Integumentary System											
Mammary gland	+	+	M	+	M	+	+	M	+	M	41
Skin	+	+	+	+	+	+	+	+	+	+	60
Keratoacanthoma											1
Subcutaneous tissue, fibrosarcoma											1
Musculoskeletal System											
None											
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	60
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar adenoma											1
Nose	+	+	+	+	+	+	+	+	+	+	60
Trachea	+	+	+	+	+	+	+	+	+	+	60
Special Senses System											
Eye											1
Zymbal's gland	+	M	+	+	+	+	+	+	+	+	59
Adenoma											1
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Urinary bladder	+	+	+	+	+	+	+	+	+	+	60
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm

Number of Days on Study	250	359	391	405	419	419	419	419	419	419	419	419	419	419	420	420	420	420	420	420	420	420	420	420	420
Carcass ID Number	31 55	29 55	30 55	36 55	29 11	29 12	29 13	29 14	30 11	30 12	30 13	30 14	31 11	31 12	31 13	31 14	32 11	32 12	32 13	32 14	32 15	33 11	33 12	33 13	33 14
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Mesentery						+					+											+	+		
Pancreas	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Tooth																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign												X													
Islets, pancreatic	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																	X								
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
Follicular cell, adenoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm (continued)

Number of Days on Study	420	420	420	420	420	420	420	420	420	421	421	421	421	421	421	421	421	421	421	421	
Carcass ID Number	335	341	342	343	344	345	351	352	353	354	355	361	362	363	364	372	373	374	375	377	Total Tissues/ Tumors
Alimentary System																					
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																					1
Mesentery							+												+		6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																					1
Tooth																			+		1
Cardiovascular System																					
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																					1
Endocrine System																					
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	44
Leukemia mononuclear																					1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pheochromocytoma benign																X					2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pars distalis, adenoma	X																				2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
C-cell, adenoma									X												1
Follicular cell, adenoma					X																1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm (continued)

Number of Days on Study	2 5 0	3 5 9	3 9 1	4 0 5	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0
Carcass ID Number	3 1 5	2 9 5	3 0 5	3 6 5	2 9 1	2 9 2	2 9 3	2 9 4	3 0 1	3 0 2	3 0 3	3 0 4	3 1 1	3 1 2	3 1 3	3 1 4	3 2 1	3 2 2	3 2 3	3 2 4	3 2 5	3 3 1	3 3 2	3 3 3	3 3 4
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X									X							
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Seminal vesicle	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Bilateral, interstitial cell, adenoma						X						X	X					X					X		
Interstitial cell, adenoma				X	X			X	X		X			X	X	X				X	X			X	
Hematopoietic System																									
Bone marrow	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, leukemia mononuclear	X																								
Mediastinal, squamous cell carcinoma, metastatic, skin			X																						
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Leukemia mononuclear	X																								
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	M	M	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																			X						X
Basal cell adenoma, multiple																X		X							
Basal cell carcinoma													X												
Keratoacanthoma																									
Leukemia mononuclear	X																								
Squamous cell carcinoma										X															
Squamous cell carcinoma, multiple			X																						
Musculoskeletal System																									
None																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm (continued)

Number of Days on Study	420	420	420	420	420	420	420	420	420	421	421	421	421	421	421	421	421	421	421	421	
Carcass ID Number	3345	3441	3442	3443	3444	3445	3551	3552	3553	3554	3555	3561	3662	3663	3664	3671	3672	3673	3674	3675	3771	**Total Tissues/ Tumors**
General Body System																						
None																						
Genital System																						
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																						1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Adenoma															X						X	4
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																						1
Bilateral, interstitial cell adenoma			X						X	X				X								9
Interstitial cell, adenoma							X						X		X				X	X	X	17
Hematopoietic System																						
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Leukemia mononuclear																						1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Mediastinal, leukemia mononuclear																						1
Mediastinal, squamous cell, carcinoma, metastatic, skin																						1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																						1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Leukemia mononuclear																						1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																						1
Thymus	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	40
Integumentary System																						
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	38
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Basal cell adenoma			X				X									X	X	X				7
Basal cell adenoma, multiple											X											3
Basal cell carcinoma																						1
Keratoacanthoma							X															1
Leukemia mononuclear																						1
Squamous cell carcinoma																						1
Squamous cell carcinoma, multiple																						1
Musculoskeletal System																						
None																						

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm (continued)

Number of Days on Study	2 5 0	3 5 9	3 9 1	4 0 5	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0
Carcass ID Number	3 1 5	2 9 5	3 0 5	3 6 5	2 9 1	2 9 2	2 9 3	2 9 4	3 0 1	3 0 2	3 0 3	3 0 4	3 1 1	3 1 2	3 1 3	3 1 4	3 2 1	3 2 2	3 2 3	3 2 4	3 2 5	3 3 1	3 3 2	3 3 3	3 3 4
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland				X																					
Squamous cell carcinoma, metastatic, skin			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																					X				
Carcinoma		X		X																					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm (continued)

Number of Days on Study	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 6	
Carcass ID Number	3 3 5	3 4 1	3 4 2	3 4 3	3 4 4	3 4 5	3 5 1	3 5 2	3 5 3	3 5 4	3 5 5	3 6 1	3 6 2	3 6 3	3 6 4	3 7 2	3 7 3	3 7 4	3 7 5	3 7 1	**Total Tissues/ Tumors**
Nervous System																					
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																					1
Respiratory System																					
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Carcinoma, metastatic, Zymbal's gland																					1
Squamous cell carcinoma, metastatic, skin																					1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																					1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Special Senses System																					
Zymbal's Gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Adenoma																					1
Carcinoma																					2
Urinary System																					
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																					1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Systemic Lesions																					
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear																					1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 5	4 2 6	4 2 6	4 2 6	
Carcass ID Number	5 4 3	5 4 4	5 4 1	5 5 2	5 5 4	5 6 1	5 6 2	5 6 3	5 6 4	5 7 1	5 8 1	5 8 2	5 8 4	5 9 1	5 9 2	5 9 3	6 0 1	6 0 2	6 1 1	6 1 2	6 1 3	5 0 5	4 9 1	4 9 2	6 0 3	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Lymphoma malignant histiocytic																										1
Polyp adenomatous																				X						1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Polyp adenomatous	X			X		X																	X			5
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Adenocarcinoma, cystic, mucinous										X												X				2
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Polyp adenomatous																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Adenocarcinoma, cystic, mucinous																					X					1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Hepatocellular carcinoma				X				X						X									X			11
Hepatocellular carcinoma, multiple							X																			1
Lymphoma malignant histiocytic																										1
Lymphoma malignant undifferentiated cell type																										1
Neoplastic nodule	X																	X	X				X			15
Neoplastic nodule, multiple				X		X	X			X				X			X					X				14
Mesentery	+			+																						6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Lymphoma malignant histiocytic																										1
Pharynx				+																				+		3
Papilloma squamous				X																				X		2
Squamous cell carcinoma																										1
Salivary glands	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Stomach,forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Tongue																										1
Papilloma squamous																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Lymphoma malignant histiocytic																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	0 1 2	2 1 5	2 2 9	2 8 5	2 8 5	2 8 5	2 9 5	3 0 7	3 4 1	3 4 3	3 5 5	3 7 2	3 7 8	3 7 9	3 7 9	3 8 4	3 8 4	3 9 1	3 9 3	4 0 4	4 0 5	4 0 5	4 0 6	4 0 6	4 0 6
Carcass ID Number	5 7 5	6 1 5	5 7 4	4 8 5	5 1 5	5 3 5	6 0 5	5 8 5	5 5 5	4 7 5	5 6 5	5 2 5	5 7 3	5 3 4	6 0 4	5 7 2	5 9 5	5 3 3	5 9 5	4 8 4	4 8 3	5 9 4	5 4 5	5 5 3	6 1 4
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X															
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma															X										
Follicular cell, adenoma																									
General Body System																									
Tissue NOS																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic											X														
Mesothelioma malignant, metastatic, testes																									
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic											X														
Mesothelioma malignant																									
Bilateral, interstitial cell, adenoma																									
Interstitial cell, adenoma									X				X						X	X	X		X		
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, lymphoma malignant histiocytic											X														
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic											X														
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	M	+	+	M	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0
Carcass ID Number	4 7 1	4 7 2	4 7 3	4 7 4	4 8 1	4 8 2	4 8 3	4 9 3	4 9 4	5 0 1	5 0 2	5 0 3	5 0 4	5 1 1	5 1 2	5 1 3	5 1 4	5 2 1	5 2 2	5 2 3	5 2 4	5 3 1	5 3 2	5 4 1	5 4 2
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma						X																			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
Follicular cell, adenoma																									
General Body System																									
Tissue NOS																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																									
Mesothelioma malignant, metastatic, testes					X																				
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma					X																				
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																									
Mesothelioma malignant					X							X													
Bilateral, interstitial cell, adenoma				X										X										X	X
Interstitial cell, adenoma	X	X						X	X		X	X				X				X	X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, lymphoma malignant histiocytic																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Thymus	M	M	+	+	+	+	+	M	+	+	+	+	+	+	M	+	M	+	+	M	+	M	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	
Carcass ID Number	5 4 3	5 4 4	5 5 1	5 5 2	5 5 4	5 6 1	5 6 2	5 6 3	5 6 4	5 7 1	5 8 1	5 8 2	5 8 4	5 9 1	5 9 2	5 9 3	4 0 1	4 0 2	4 1 1	4 1 2	4 1 3	4 5 5	4 9 1	4 9 2	6 0 3	**Total Tissues/ Tumors**
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Pheochromocytoma benign																			X							1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	71
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Pars distalis, adenoma														X	X											4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
C-cell, adenoma			X													X										3
Follicular cell, adenoma				X																						1
General Body System																										
Tissue NOS													+													1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Lymphoma malignant histiocytic																										1
Mesothelioma malignant, metastatic testes															X											2
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Adenoma	X				X									X									X			4
Carcinoma						X																				2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Lymphoma malignant histiocytic																										1
Mesothelioma malignant															X											3
Bilateral,interstitial cell, adenoma																						X		X		6
Interstitial cell, adenoma						X		X	X					X			X									20
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Mediastinal, lymphoma malignant histiocytic																										1
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Lymphoma malignant histiocytic																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Leukemia mononuclear													X													1
Thymus	+	+	+	+	+	M	+	+	M	M	+	M	M	M	+	M	M	+	M	M	M	+	M	M	M	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	0 1 2	2 1 5	2 2 9	2 8 5	2 8 5	2 8 5	2 9 5	3 0 7	3 0 4 1	3 4 1 3	3 5 3 5	3 5 2	3 7 7 8	3 7 9	3 7 9	3 8 4	3 8 4	3 9 1	3 9 3	4 0 4	4 0 5	4 0 5	4 0 6	4 0 6	4 0 6
Carcass ID Number	5 7 5	6 1 5	5 7 4	4 8 5	5 1 5	5 3 5	6 0 5	5 8 5	5 5 5	4 7 5	5 6 5	5 2 5	5 7 3	5 3 4	6 0 4	5 7 2	5 9 5	5 3 3	4 9 5	4 8 4	5 8 3	5 9 4	5 4 5	5 5 3	6 1 4
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma								X			X					X			X		X			X	X
Basal cell adenoma, multiple												X			X		X	X		X			X		
Basal cell carcinoma																									
Keratoacanthoma																									
Keratoacanthoma, multiple														X											
Papilloma squamous																					X				X
Squamous cell carcinoma																						X			
Squamous cell carcinoma, multiple																									
Sebaceous gland, adenoma																					X	X			
Sebaceous gland,adenoma, multiple																									
Subcutaneous tissue, schwannoma benign																									
Musculoskeletal System																									
None																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland																			X						
Glioma malignant																									
Lymphoma malignant histiocytic											X														
Meninges, sarcoma													X												
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																						X			
Alveolar/bronchiolar adenoma, multiple																									
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, Zymbal's gland												X							X						
Lymphoma malignant histiocytic											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	419	419	419	419	419	419	419	419	419	419	419	419	419	419	419	419	420	420	420	420	420	420	420	420	420
Carcass ID Number	47101	47202	47303	47404	48101	48202	48303	49303	49404	50101	50202	50303	50404	50111	51202	51303	51401	52201	52302	52403	53101	53202	54101	54202
Integumentary System																									
Mammary gland	M	+	M	M	M	+	+	+	+	+	M	M	+	+	+	M	M	M	+	+	+	+	+	+	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma				X													X			X	X				
Basal cell adenoma, multiple	X	X	X			X	X			X	X	X	X		X	X		X				X	X	X	X
Basal cell carcinoma																								X	
Keratoacanthoma		X									X				X										X
Keratoacanthoma, multiple																									
Papilloma squamous		X									X													X	
Squamous cell carcinoma								X	X										X	X					
Squamous cell carcinoma, multiple															X										
Sebaceous gland, adenoma																				X					
Sebaceous gland, adenoma, multiple																									
Subcutaneous tissue, schwannoma benign				X																					
Musculoskeletal System																									
None																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland																									
Glioma malignant																									
Lymphoma malignant histiocytic																									
Meninges, sarcoma																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X			X																		
Alveolar/bronchiolar adenoma, multiple																						X			
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, Zymbal's gland																									
Lymphoma malignant histiocytic																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 5	4 2 6	4 2 6	4 2 6	
Carcass ID Number	5 4 3	5 4 4	5 4 1	5 5 2	5 5 4	5 6 1	5 6 2	5 6 3	5 6 4	5 7 1	5 8 1	5 8 2	5 8 4	5 9 1	5 9 2	5 9 3	6 0 1	6 0 2	6 1 1	6 1 2	6 1 3	5 0 5	4 9 1	4 9 2	6 0 3	**Total Tissues/ Tumors**
Integumentary System																										
Mammary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	61
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Basal cell adenoma	X						X				X					X			X						X	17
Basal cell adenoma, multiple		X		X	X					X				X	X		X	X		X	X	X	X	X		35
Basal cell carcinoma						X		X															X			4
Keratoacanthoma						X			X															X		7
Keratoacanthoma, multiple																										1
Papilloma squamous				X													X						X			8
Squamous cell carcinoma				X		X						X														8
Squamous cell carcinoma, multiple															X											2
Sebaceous gland, adenoma									X							X			X							6
Sebaceous gland, adenoma, multiple																								X		1
Subcutaneous tissue, schwannoma benign																										1
Musculoskeletal System																										
None																										
Nervous system																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Carcinoma, metastatic, Zymbal's gland																	X									1
Glioma malignant																X										1
Lymphoma malignant histiocytic																										1
Meninges, sarcoma																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Alveolar/bronchiolar adenoma			X					X	X																	6
Alveolar/bronchiolar adenoma, multiple																										1
Alveolar/bronchiolar carcinoma																X										1
Carcinoma, metastatic, Zymbal's gland																								X		3
Lymphoma malignant histiocytic																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	0 1 2	2 1 5	2 2 9	2 8 5	2 8 5	2 8 5	2 9 5	3 0 7	3 4 1	3 4 3	3 4 5	3 5 2	3 7 8	3 7 9	3 7 9	3 8 4	3 8 4	3 9 1	3 9 3	4 0 4	4 0 5	4 0 5	4 0 6	4 0 6	4 0 6
Carcass ID Number	5 7 5	6 1 5	5 7 4	4 8 5	5 1 5	5 3 5	6 0 5	5 8 5	5 5 5	4 7 5	5 6 5	5 2 5	5 7 3	5 3 4	6 0 4	5 7 2	5 9 5	5 3 3	5 9 5	4 8 4	5 8 3	5 9 4	5 4 5	5 5 3	6 1 4
Special Senses System																									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X		X							X			
Carcinoma			X	X	X	X	X			X		X		X	X	X	X	X	X	X	X				
Bilateral, carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									
Lymphoma malignant histiocytic											X														
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Lymphoma malignant histiocytic											X														
Lymphoma malignant undifferentiated cell type																									
Mesothelioma malignant																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 1 9	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0	4 2 0
Carcass ID Number	4 7 1	4 7 2	4 7 3	4 7 4	4 8 1	4 8 2	4 8 3	4 9 3	4 9 4	5 0 1	5 0 2	5 0 3	5 0 4	5 1 1	5 1 2	5 1 3	5 1 4	5 2 1	5 2 2	5 2 3	5 2 4	5 3 1	5 3 2	5 4 1	5 4 2
Special Senses System																									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X								X			X		X	
Carcinoma								X	X																
Bilateral, carcinoma				X																					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																X									
Lymphoma malignant histiocytic																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Lymphoma malignant histiocytic																									
Lymphoma malignant undifferentiated cell type							X																		
Mesothelioma malignant					X							X													

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	420	420	420	420	420	420	420	420	420	420	421	421	421	421	421	421	421	421	421	421	421	426	426	426	
Carcass ID Number	54 3	44 4	55 1	55 2	55 4	56 1	66 2	66 3	66 4	67 1	58 1	58 2	58 4	59 1	59 2	59 3	60 1	60 2	61 1	61 2	61 3	10 5	49 1	49 2	60 3	**Total Tissues/ Tumors**
Special Senses System																										
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Adenoma			X								X		X	X					X			X				13
Carcinoma											X							X						X		20
Bilateral, carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Lipoma																										1
Lymphoma malignant histiocytic																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Leukemia mononuclear													X													1
Lymphoma malignant histiocytic																										1
Lymphoma malignant undifferentiated cell type																										1
Mesothelioma malignant															X											3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm

Number of Days on Study	209	211	116	269	238	354	464	464	465	468	480	488	488	488	488	491	490	496	493	503	506	508	508	509
Carcass ID Number	845	775	905	844	744	853	951	114	775	743	735	504	434	373	404	455	553	533	654	544	443	024	434	044
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																							X	
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																								
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																								
Adenocarcinoma, cystic, mucinous																								
Polyp adenomatous																							X	X
Polyp adenomatous, multiple																								
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																								
Polyp adenomatous																								
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Adenocarcinoma, multiple																								
Polyp adenomatous																								
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Adenocarcinoma, cystic, mucinous			X				X										X			X				
Adenocarcinoma, cystic, mucinous, multiple																								
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cholangiocarcinoma																								
Hepatoblastoma																								
Hepatocellular carcinoma						X														X				
Hepatocellular carcinoma, multiple																								
Leukemia mononuclear																							X	
Neoplastic nodule													X									X	X	X
Neoplastic nodule, multiple																X								
Mesentery			+								+											+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Leukemia mononuclear																							X	
Pharynx																								
Papilloma squamous																								
Squamous cell carcinoma																								
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																								
Squamous cell carcinoma																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	3 5 2	3 5 2	3 5 2	3 5 2	3 7 2	3 7 2	3 7 2	3 7 9	3 8 3	3 8 4	
Carcass ID Number	8 8 2	8 8 3	8 9 2	8 9 3	8 6 1	8 8 1	8 9 1	7 9 4	9 0 1	8 3 1	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	60
Intestine large	+	+	+	+	+	+	+	+	+	+	60
Adenocarcinoma											1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	58
Polyp adenomatous											1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	57
Adenocarcinoma											4
Adenocarcinoma, cystic, mucinous											1
Polyp adenomatous											3
Polyp adenomatous, multiple											2
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	60
Adenocarcinoma											1
Polyp adenomatous					X		X				3
Intestine small	+	+	+	+	+	+	+	+	+	+	56
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	56
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	54
Adenocarcinoma, multiple									X		1
Polyp adenomatous									X		1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	55
Adenocarcinoma, cystic, mucinous		X									6
Adenocarcinoma, cystic, mucinous multiple											1
Liver	+	+	+	+	+	+	+	+	+	+	60
Cholangiocarcinoma											1
Hepatoblastoma											1
Hepatocellular carcinoma					X						10
Hepatocellular carcinoma, multiple									X		2
Leukemia mononuclear											1
Neoplastic nodule					X		X	X	X		15
Neoplastic nodule, multiple	X			X						X	11
Mesentery											7
Pancreas	+	+	+	+	+	+	+	+	+	+	57
Leukemia mononuclear											1
Pharynx								+			3
Papilloma squamous								X			2
Squamous cell carcinoma											1
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Stomach	+	+	+	+	+	+	+	+	+	+	59
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	59
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	59
Tongue	+										3
Squamous cell carcinoma	X										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	3 5 2	3 5 2	3 5 2	3 5 2	3 7 2	3 7 2	3 7 2	3 7 9	3 8 3	3 8 4	
Carcass ID Number	8 8 2	8 8 3	8 9 2	8 9 3	8 6 1	8 8 1	8 9 1	7 9 4	9 0 1	8 3 1	**Total Tissues/ Tumors**
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	59
Leukemia mononuclear											1
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	59
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	59
Leukemia mononuclear											1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	59
Leukemia mononuclear											1
Pheochromocytoma benign									X		3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	57
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	58
Pituitary gland	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Pars distalis, adenoma											1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	60
C-cell, adenoma										X	1
Follicular cell, adenoma											1
General Body System											
Tissue NOS											4
Sarcoma											1
Genital System											
Epididymis	+	+	+	+	+	+	+	+	+	+	60
Mesothelioma malignant											1
Mesothelioma malignant, metastatic testes		X						X	X		3
Preputial gland	+	+	+	+	+	+	+	+	+	+	60
Adenoma						X					8
Carcinoma											1
Prostate	+	+	+	+	+	+	+	+	+	+	60
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	58
Testes	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Mesothelioma malignant		X						X	X		3
Bilateral, interstitial cell adenoma		X									1
Interstitial cell, adenoma											1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	2 0 9	2 1 1	2 1 1	2 2 6	2 2 9	2 3 8	2 5 4	2 6 4	2 6 4	2 6 5	2 6 8	2 8 0	2 8 0	2 8 0	2 8 0	2 8 1	2 9 0	2 9 6	2 9 6	3 0 3	3 0 5	3 0 6	3 0 8	3 0 8	3 0 9
Carcass ID Number	8 4 5	8 7 5	8 7 5	8 0 4	8 4 4	8 7 5	8 1 5	8 1 4	8 7 5	8 7 4	8 7 3	8 0 5	8 3 4	8 7 3	8 9 0	8 0 4	8 2 5	8 5 5	8 3 3	8 6 5	8 5 4	8 6 4	8 9 0 3	8 0 2 4	8 4 3	8 0 4
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								X	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, leukemia mononuclear																								X	
Pancreatic, leukemia mononuclear																								X	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic skin												X													
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+
Fibroma																						X			
Leukemia mononuclear																								X	
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																	X				X		X		
Basal cell adenoma, multiple																X									
Basal cell carcinoma																			X						
Keratoacanthoma																									
Papilloma squamous						X											X		X						X
Papilloma squamous, multiple																									
Squamous cell carcinoma			X									X												X	
Squamous cell carcinoma, multiple																									
Sebaceous gland, adenoma														X											
Sebaceous gland, adenoma, multiple																									
Subcutaneous tissue, fibroma																									
Musculoskeletal System																									
Bone																							+		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	3 0 9	3 1 0	3 1 2	3 2 4	3 2 4	3 2 4	3 2 8	3 3 3	3 3 8	3 3 8	3 3 8	3 3 8	3 3 8	3 3 8	3 3 8	3 4 3	3 4 3	3 4 4	3 4 8	3 4 9	3 5 0	3 5 0	3 5 2	3 5 2	3 5 2
Carcass ID Number	8 7 2	8 1 3	8 0 3	8 3 2	8 8 5	8 9 5	8 7 1	8 1 2	8 0 1	8 0 2	8 2 3	8 5 3	8 6 3	8 8 4	9 0 2	8 5 2	8 6 2	7 9 5	8 5 1	8 9 4	8 2 1	8 4 1	8 1 1	8 2 2	8 4 2
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, leukemia mononuclear																									
Pancreatic, leukemia mononuclear																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic skin																									
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+
Fibroma																									
Leukemia mononuclear																									
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	M	+	M	M
Integumentary System																									
Mammary gland	+	+	M	M	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma					X				X					X					X						
Basal cell adenoma, multiple						X	X			X		X	X					X			X	X	X	X	X
Basal cell carcinoma									X																
Keratoacanthoma														X				X							
Papilloma squamous			X		X												X		X						
Papilloma squamous, multiple				X																				X	
Squamous cell carcinoma	X						X				X		X	X	X							X			
Squamous cell carcinoma, multiple												X													
Sebaceous gland, adenoma														X							X				
Sebaceous gland, adenoma, multiple												X													
Subcutaneous tissue, fibroma																					X				
Musculoskeletal System																									
Bone									+	+									+			+			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	3 5 2	3 5 2	3 5 2	3 5 2	3 7 2	3 7 2	3 7 2	3 7 9	3 8 3	3 8 4	
Carcass ID Number	8 8 2	8 8 3	8 9 2	8 9 3	8 6 1	8 8 1	8 9 1	7 9 4	9 0 1	8 3 1	**Total Tissues/ Tumors**
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Lymph node	+	+	+	+	+	+	+	+	+	+	60
Mediastinal, leukemia mononuclear											1
Pancreatic, leukemia mononuclear											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	60
Squamous cell carcinoma, metastatic skin											1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	56
Spleen	+	+	+	+	+	+	+	+	+	+	55
Fibroma											1
Leukemia mononuclear											1
Thymus	+	+	+	M	M	+	M	+	+	+	48
Integumentary System											
Mammary gland	+	+	M	+	M	+	M	M	+	M	48
Skin	+	+	+	+	+	+	+	+	+	+	60
Basal cell adenoma									X		8
Basal cell adenoma, multiple	X	X	X	X	X	X	X	X		X	21
Basal cell carcinoma											2
Keratoacanthoma			X			X	X				5
Papilloma squamous	X				X	X		X		X	13
Papilloma squamous, multiple											2
Squamous cell carcinoma			X								10
Squamous cell carcinoma, multiple							X	X			3
Sebaceous gland, adenoma				X							4
Sebaceous gland, adenoma, multiple											1
Subcutaneous tissue, fibroma										X	2
Musculoskeletal System											
Bone											5

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	2 0 9	2 1 1	2 1 1	2 2 6	2 2 9	2 3 8	2 5 4	2 6 4	2 6 4	2 6 5	2 6 8	2 8 0	2 8 0	2 8 0	2 8 0	2 8 1	2 9 0	2 9 6	2 9 6	3 0 3	3 0 5	3 0 6	3 0 8	3 0 8	3 0 9
Carcass ID Number	8 4 5	7 7 5	9 0 5	8 4 4	7 7 4	8 3 5	8 1 5	8 1 4	8 7 5	8 7 4	7 7 3	8 0 5	8 3 4	8 7 3	9 0 4	8 2 5	8 5 5	8 3 3	8 6 5	8 5 4	8 6 4	9 0 3	8 2 4	8 4 3	8 0 4
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant, focal, mild																									
Leukemia mononuclear																								X	
Meningioma malignant																									
Cranial nerve, meninges, carcinoma, metastatic, Zymbal's gland																					X				
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X	X										X										
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic, Zymbal's gland	X																			X					X
Leukemia mononuclear																								X	
Squamous cell carcinoma, metastatic, skin						X																			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Squamous cell carcinoma																									
Canal, papilloma squamous																									
Eye																					+				
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X									X	X								
Carcinoma	X	X		X	X			X	X	X			X	X	X			X		X	X			X	
Bilateral, adenoma																									
Bilateral, carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Leukemia mononuclear																								X	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								X	
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								X	
Mesothelioma malignant																								X	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	309	311	310	312	322	324	322	333	338	338	338	338	338	338	338	343	344	344	348	349	350	350	352	352	352
Carcass ID Number	872	871	873	870	873	875	879	877	871	870	870	872	873	873	876	880	882	885	876	895	892	894	891	911	912
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma malignant, focal, mild																				X					
Leukemia mononuclear																									
Meningioma malignant														X											
Cranial nerve, meninges, carcinoma metastatic, Zymbal's gland																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar adenoma, multiple						X																			
Carcinoma, metastatic, Zymbal's gland																									
Leukemia mononuclear																									
Squamous cell carcinoma, metastatic skin											X		X												
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																			+	+					
Squamous cell carcinoma																			X						
Canal, papilloma squamous																				X					
Eye				+																		+			
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Adenoma	X						X	X		X							X								X
Carcinoma	X	X										X				X	X				X				
Bilateral, adenoma									X													X			
Bilateral, carcinoma																							X		
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
Leukemia mononuclear																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Mesothelioma malignant																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	3 3 3 3 3 3 3 3 3 3 5 5 5 5 7 7 7 7 8 8 2 2 2 2 2 2 2 9 3 4	
Carcass ID Number	8 8 8 8 8 8 8 7 9 8 8 8 9 9 6 8 9 9 0 3 2 3 2 3 1 1 1 4 1 1	**Total Tissues/ Tumors**
Nervous System		
Brain	+ + + + + + + + + +	60
Glioma malignant, focal, mild		1
Leukemia mononuclear		1
Meningioma malignant		1
Cranial nerve, meninges, carcinoma metastatic, Zymbal's gland		1
Respiratory System		
Lung	+ + + + + + + + + +	60
Alveolar/bronchiolar adenoma	X	4
Alveolar/bronchiolar adenoma, multiple	X	2
Carcinoma, metastatic, Zymbal's gland		3
Leukemia mononuclear		1
Squamous cell carcinoma, metastatic skin		3
Nose	+ + + + + + + + + +	60
Trachea	+ + + + + + + + + +	60
Special Senses System		
Ear		2
Squamous cell carcinoma		1
Canal, papilloma squamous		1
Eye	+	4
Zymbal's gland	+ + + + + + + + + +	59
Adenoma	X X X X	13
Carcinoma	X X	22
Bilateral, adenoma	X	3
Bilateral, carcinoma		1
Urinary System		
Kidney	+ + + + + + + + + +	59
Leukemia mononuclear		1
Urinary bladder	+ + + + + + + + + +	59
Leukemia mononuclear		1
Systemic lesions		
Multiple organs	+ + + + + + + + + +	60
Leukemia mononuclear		1
Mesothelioma malignant	X X X	4

TABLE A3
Statistical Analysis of Primary Tumors in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rates[a]	0/60 (0%)	2/45 (4%)	1/75 (1%)	3/59 (5%)
Effective rates[b]	0/60 (0%)	2/44 (5%)	1/65 (2%)	3/16 (19%)
Terminal rates[c]	0/60 (0%)	2/41 (5%)	1/50 (2%)	0/0
First incidence (days)		419 (T)	419 (T)	349
Life table tests[d]	P<0.001	P=0.160	P=0.464	P<0.001
Logistic regression tests[d]	P=0.029	P=0.160	P=0.464	P=0.024
Cochran-Armitage test[d]	P=0.005			
Fisher exact test[d]		P=0.177	P=0.520	P=0.008
Preputial Gland: Adenoma				
Overall rates	2/60 (3%)	4/45 (9%)	4/75 (5%)	8/60 (13%)
Effective rates	2/60 (3%)	4/44 (9%)	4/72 (6%)	8/49 (16%)
Terminal rates	2/60 (3%)	4/41 (10%)	4/50 (8%)	0/0
First incidence (days)	419 (T)	419 (T)	419 (T)	280
Life table tests	P<0.001	P=0.182	P=0.258	P<0.001
Logistic regression tests	P=0.016	P=0.182	P=0.258	P=0.036
Cochran-Armitage test	P=0.018			
Fisher exact test		P=0.206	P=0.430	P=0.022
Preputial Gland: Adenoma or Carcinoma				
Overall rates	2/60 (3%)	4/45 (9%)	6/75 (8%)	9/60 (15%)
Effective rates	2/60 (3%)	4/44 (9%)	6/72 (8%)	9/49 (18%)
Terminal rates	2/60 (3%)	4/41 (10%)	6/50 (12%)	0/0
First incidence (days)	419 (T)	419 (T)	419 (T)	280
Life table tests	P<0.001	P=0.182	P=0.086	P<0.001
Logistic regression tests	P=0.006	P=0.182	P=0.086	P=0.030
Cochran-Armitage test	P=0.008			
Fisher exact test		P=0.206	P=0.205	P=0.011
Large Intestine: Adenomatous Polyp				
Overall rates	0/60 (0%)	0/45 (0%)	6/75 (8%)	9/60 (15%)
Effective rates	0/60 (0%)	0/44 (0%)	6/67 (9%)	9/38 (24%)
Terminal rates	0/60 (0%)	0/41 (0%)	5/50 (10%)	0/0 (0%)
First incidence (days)			384	308
Life table tests	P<0.001	–[e]	P=0.012	P<0.001
Logistic regression tests	P<0.001	–[e]	P=0.020	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	P=0.019	P<0.001
Large Intestine: Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	0/75 (0%)	7/60 (12%)
Effective rates	0/60 (0%)	0/44 (0%)	0/67 (0%)	7/36 (19%)
Terminal rates	0/60 (0%)	0/41 (0%)	0/50 (0%)	0/0
First incidence (days)				309
Life table tests	P<0.001	–[e]	–[e]	P<0.001
Logistic regression tests	P<0.001	–[e]	–[e]	P=0.003
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	–[e]	P<0.001

TABLE A3
Statistical Analysis of Primary Tumors in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Large Intestine: Adenomatous Polyp or Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	6/75 (8%)	15/60 (25%)
Effective rates	0/60 (0%)	0/45 (0%)	6/67 (9%)	15/38 (39%)
Terminal rates	0/60 (0%)	0/41 (0%)	5/50 (10%)	0/0 (0%)
First incidence (days)			384	308
Life table tests	P<0.001	–[e]	P=0.012	P<0.001
Logistic regression tests	P<0.001	–[e]	P=0.020	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	P=0.019	P<0.001
Large Intestine (Colon): Adenomatous Polyp				
Overall	0/60 (0%)	0/45 (0%)	5/75 (7%)	5/60 (8%)
Effective rates	0/60 (0%)	0/44 (0%)	5/67 (7%)	5/38 (13%)
Terminal rates	0/60 (0%)	0/41 (0%)	4/50 (8%)	0/0
First incidence (days)			384	308
Life table tests	P<0.001	–[e]	P=0.024	P<0.001
Logistic regression tests	P=0.005	–[e]	P=0.039	P=0.046
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	P=0.038	P=0.007
Large Intestine (Colon): Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	0/75 (0%)	5/60 (8%)
Effective rates	0/60 (0%)	0/44 (0%)	0/67 (0%)	5/36 (14%)
Terminal rates	0/60 (0%)	0/41 (0%)	0/50 (0%)	0/0
First incidence (days)				309
Life table tests	P<0.001	–[e]	–[e]	P<0.001
Logistic regression tests	P=0.004	–[e]	–[e]	P=0.024
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	–[e]	P=0.006
Large Intestine (Rectum): Adenomatous Polyp				
Overall rates	0/60 (0%)	0/45 (0%)	0/75 (0%)	3/60 (5%)
Effective rates	0/60 (0%)	0/44 (0%)	0/67 (0%)	3/27 (11%)
Terminal rates	0/60 (0%)	0/41 (0%)	0/50 (0%)	0/0
First incidence (days)				338
Life table tests	P<0.001	–[e]	–[e]	P<0.001
Logistic regression tests	P=0.014	–[e]	–[e]	P=0.026
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	–[e]	P=0.028
Small Intestine: Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	3/75 (4%)	8/60 (13%)
Effective rates	0/60 (0%)	0/45 (0%)	3/74 (4%)	8/59 (14%)
Terminal rates	0/60 (0%)	0/41 (0%)	3/50 (6%)	0/0
First incidence (days)			419 (T)	211
Life table tests	P<0.001	–[e]	P=0.092	P<0.001
Logistic regression tests	P=0.003	–[e]	P=0.092	P=0.096
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	P=0.165	P=0.003

TABLE A3
Statistical Analysis of Primary Tumors in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Small Intestine: Adenomatous Polyp or Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	4/75 (5%)	8/60 (13%)
Effective rates	0/60 (0%)	0/45 (0%)	4/74 (5%)	8/59 (14%)
Terminal rates	0/60 (0%)	0/41 (0%)	3/50 (6%)	0/0 (0%)
First incidence (days)			379	211
Life table tests	P<0.001	–[e]	P=0.050	P<0.001
Logistic regression tests	P=0.004	–[e]	P=0.078	P=0.096
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	P=0.090	P=0.003
Small Intestine (Jejunum): Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	1/75 (1%)	7/60 (12%)
Effective rates	0/60 (0%)	0/45 (0%)	1/74 (1%)	7/59 (12%)
Terminal rates	0/60 (0%)	0/41 (0%)	1/50 (2%)	0/0
First incidence (days)			419 (T)	211
Life table tests	P<0.001	–[e]	P=0.464	P<0.001
Logistic regression tests	P=0.006	–[e]	P=0.464	P=0.247
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	P=0.552	P=0.006
Liver: Neoplastic Nodule				
Overall rates	0/60 (0%)	0/45 (0%)	29/75 (39%)	26/60 (43%)
Effective rates	0/60 (0%)	0/44 (0%)	29/72 (40%)	26/49 (53%)
Terminal rates	0/60 (0%)	0/41 (0%)	23/50 (46%)	0/0
First incidence (days)			393	280
Life table tests	P<0.001	–[e]	P<0.001	P<0.001
Logistic regression tests	P<0.001	–[e]	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	12/75 (16%)	12/60 (20%)
Effective rates	0/60 (0%)	0/45 (0%)	12/72 (17%)	12/55 (22%)
Terminal rates	0/60 (0%)	0/41 (0%)	11/50 (22%)	0/0
First incidence (days)			379	238
Life table tests	P<0.001	–[e]	P<0.001	P<0.001
Logistic regression tests	P<0.001	–[e]	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	P<0.001	P<0.001
Liver: Neoplastic Nodule or Hepatocellular Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	35/75 (47%)	33/60 (55%)
Effective rates	0/60 (0%)	0/45 (0%)	35/72 (49%)	33/55 (60%)
Terminal rates	0/60 (0%)	0/41 (0%)	28/50 (56%)	0/0
First incidence (days)			379	238
Life table tests	P<0.001	–[e]	P<0.001	P<0.001
Logistic regression tests	P<0.001	–[e]	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	P<0.001	P<0.001

TABLE A3
Statistical Analysis of Primary Tumors in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Lung: Alveolar/Bronchiolar Adenoma				
Overall rates	1/60 (2%)	0/45 (0%)	7/75 (9%)	6/60 (10%)
Effective rates	1/60 (2%)	0/45 (0%)	7/73 (10%)	6/57 (11%)
Terminal rates	1/60 (2%)	0/41 (0%)	6/50 (12%)	0/0
First incidence (days)	419 (T)		406	226
Life table tests	P<0.001	P=0.576N	P=0.019	P<0.001
Logistic regression tests	P=0.025	P=0.576N	P=0.026	P=0.472
Cochran-Armitage test	P=0.012			
Fisher exact test		P=0.571N	P=0.057	P=0.049
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma				
Overall rates	1/60 (2%)	0/45 (0%)	8/75 (11%)	6/60 (10%)
Effective rates	1/60 (2%)	0/45 (0%)	8/73 (11%)	6/57 (11%)
Terminal rates	1/60 (2%)	0/41 (0%)	7/50 (14%)	0/0
First incidence (days)	419 (T)		406	226
Life table tests	P<0.001	P=0.576N	P=0.010	P<0.001
Logistic regression tests	P=0.019	P=0.576N	P=0.013	P=0.472
Cochran-Armitage test	P=0.013			
Fisher exact test		P=0.571N	P=0.033	P=0.049
Oral Cavity (Tongue and Pharynx): Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	1/75 (1%)	3/60 (5%)
Effective rates	0/60 (0%)	0/44 (0%)	1/67 (1%)	3/32 (9%)
Terminal rates	0/60 (0%)	0/41 (0%)	0/50 (0%)	0/0
First incidence (days)			341	324
Life table tests	P=0.001	–[e]	P=0.522	P=0.015
Logistic regression tests	P=0.075	–[e]	P=0.650	P=0.159
Cochran-Armitage test	P=0.004			
Fisher exact test		–[e]	P=0.528	P=0.039
Oral Cavity (Tongue and Pharynx): Squamous Cell Papilloma or Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	4/75 (5%)	5/60 (8%)
Effective rates	0/60 (0%)	0/44 (0%)	4/67 (6%)	5/32 (16%)
Terminal rates	0/60 (0%)	0/41 (0%)	3/50 (6%)	0/0
First incidence (days)			341	324
Life table tests	P<0.001	–[e]	P=0.052	P<0.001
Logistic regression tests	P=0.007	–[e]	P=0.094	P=0.017
Cochran-Armitage test	P<0.001			
Fisher exact test		–[e]	P=0.074	P=0.004
Pituitary Gland (Pars Distalis): Adenoma				
Overall rates	1/60 (2%)	2/45 (4%)	4/75 (5%)	1/60 (2%)
Effective rates	1/60 (2%)	2/44 (5%)	4/68 (6%)	1/41 (2%)
Terminal rates	1/60 (2%)	2/41 (5%)	3/50 (6%)	0/0
First incidence (days)	419 (T)	419 (T)	343	303
Life table tests	P=0.057	P=0.369	P=0.147	P=0.424
Logistic regression tests	P=0.515	P=0.369	P=0.246	P=0.875N
Cochran-Armitage test	P=0.525			
Fisher exact test		P=0.384	P=0.224	P=0.650

TABLE A3
Statistical Analysis of Primary Tumors in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Skin: Basal Cell Adenoma				
Overall rates	0/60 (0%)	10/45 (22%)	52/75 (69%)	29/60 (48%)
Effective rates	0/60 (0%)	10/44 (23%)	52/72 (72%)	29/45 (64%)
Terminal rates	0/60 (0%)	10/41 (24%)	39/50 (78%)	0/0
First incidence (days)		419 (T)	307	281
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
Skin: Keratoacanthoma				
Overall rates	1/60 (2%)	1/45 (2%)	8/75 (11%)	5/60 (8%)
Effective rates	1/60 (2%)	1/44 (2%)	8/67 (12%)	5/27 (19%)
Terminal rates	1/60 (2%)	1/41 (2%)	7/50 (14%)	0/0
First incidence (days)	419 (T)	419 (T)	379	338
Life table tests	P<0.001	P=0.674	P=0.010	P<0.001
Logistic regression tests	P<0.001	P=0.674	P=0.020	P=0.008
Cochran-Armitage test	P=0.001			
Fisher exact test		P=0.670	P=0.024	P=0.010
Skin: Basal Cell Carcinoma				
Overall rates	0/60 (0%)	1/45 (2%)	4/75 (5%)	2/60 (3%)
Effective rates	0/60 (0%)	1/44 (2%)	4/68 (6%)	2/43 (5%)
Terminal rates	0/60 (0%)	1/41 (2%)	4/50 (8%)	0/0
First incidence (days)		419 (T)	419 (T)	296
Life table tests	P<0.001	P=0.424	P=0.043	P=0.127
Logistic regression tests	P=0.075	P=0.424	P=0.043	P=0.577
Cochran-Armitage test	P=0.121			
Fisher exact test		P=0.423	P=0.076	P=0.172
Skin: Squamous Cell Papilloma				
Overall rates	0/60 (0%)	0/45 (0%)	8/75 (11%)	15/60 (25%)
Effective rates	0/60 (0%)	0/45 (0%)	8/72 (11%)	15/55 (27%)
Terminal rates	0/60 (0%)	0/41 (0%)	6/50 (12%)	0/0
First incidence (days)			405	238
Life table tests	P<0.001	-[e]	P=0.003	P<0.001
Logistic regression tests	P<0.001	-[e]	P=0.005	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		-[e]	P=0.006	P<0.001
Skin: Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	2/45 (4%)	10/75 (13%)	13/60 (22%)
Effective rates	0/60 (0%)	2/45 (4%)	10/74 (14%)	13/59 (22%)
Terminal rates	0/60 (0%)	1/41 (2%)	9/50 (18%)	0/0
First incidence (days)		391	406	211
Life table tests	P<0.001	P=0.165	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.202	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P=0.002	P<0.001

TABLE A3
Statistical Analysis of Primary Tumors in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Skin: Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	2/45 (4%)	17/75 (23%)	27/60 (45%)
Effective rates	0/60 (0%)	2/45 (4%)	17/74 (23%)	27/59 (46%)
Terminal rates	0/60 (0%)	1/41 (2%)	14/50 (28%)	0/0
First incidence (days)		391	405	211
Life table tests	P<0.001	P=0.165	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.202	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P<0.001	P<0.001
Skin: Basal Cell Adenoma or Basal Cell Carcinoma				
Overall rates	0/60 (0%)	11/45 (24%)	54/75 (72%)	30/60 (50%)
Effective rates	0/60 (0%)	11/44 (25%)	54/72 (75%)	30/45 (67%)
Terminal rates	0/60 (0%)	11/41 (27%)	41/50 (82%)	0/0
First incidence (days)		419 (T)	307	281
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
Skin (Sebaceous Gland): Adenoma				
Overall rates	0/60 (0%)	0/45 (0%)	7/75 (9%)	5/60 (8%)
Effective rates	0/60 (0%)	0/44 (0%)	7/72 (10%)	5/49 (10%)
Terminal rates	0/60 (0%)	0/41 (0%)	5/50 (10%)	0/0
First incidence (days)			405	280
Life table tests	P<0.001	–[e]	P=0.006	P=0.001
Logistic regression tests	P=0.003	–[e]	P=0.010	P=0.058
Cochran-Armitage test	P=0.005			
Fisher exact test		–[e]	P=0.012	P=0.016
Testes: Adenoma				
Overall rates	24/60 (40%)	26/45 (58%)	26/75 (35%)	2/60 (3%)
Effective rates	24/60 (40%)	26/44 (59%)	26/67 (39%)	2/20 (10%)
Terminal rates	24/60 (40%)	25/41 (61%)	20/50 (40%)	0/0
First incidence (days)	419 (T)	405	341	348
Life table tests	P=0.043	P=0.020	P=0.186	P=0.026
Logistic regression tests	P=0.453N	P=0.025	P=0.497	P=0.802N
Cochran-Armitage test	P=0.008N			
Fisher exact test		P=0.042	P=0.517N	P=0.010N
Zymbal's Gland: Adenoma				
Overall rates	1/60 (2%)	1/45 (2%)	13/75 (17%)	16/60 (27%)
Effective rates	1/60 (2%)	1/44 (2%)	13/72 (18%)	16/54 (30%)
Terminal rates	1/60 (2%)	1/41 (2%)	10/50 (20%)	0/0
First incidence (days)	419 (T)	419 (T)	378	254
Life table tests	P<0.001	P=0.674	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.674	P=0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.670	P=0.002	P<0.001

TABLE A3
Statistical Analysis of Primary Tumors in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Zymbal's Gland: Carcinoma				
Overall rates	0/60 (0%)	2/45 (4%)	21/75 (28%)	23/60 (38%)
Effective rates	0/60 (0%)	2/45 (4%)	21/74 (28%)	23/60 (38%)
Terminal rates	0/60 (0%)	0/41 (0%)	6/50 (12%)	0/0
First incidence (days)		359	229	209
Life table tests	P<0.001	P=0.170	P<0.001	P<0.001
Logistic regression tests	P=0.009	P=0.265	P<0.001	P=0.056
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P<0.001	P<0.001
Zymbal's Gland: Adenoma or Carcinoma				
Overall rates	1/60 (2%)	3/45 (7%)	32/75 (43%)	36/60 (60%)
Effective rates	1/60 (2%)	3/45 (7%)	32/74 (43%)	36/60 (60%)
Terminal rates	1/60 (2%)	1/41 (2%)	15/50 (30%)	0/0
First incidence (days)	419 (T)	359	229	209
Life table tests	P<0.001	P=0.192	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.280	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.209	P<0.001	P<0.001
All Organs: Mesothelioma (Benign, Malignant, NOS)				
Overall rates	0/60 (0%)	0/45 (0%)	3/75 (4%)	4/60 (7%)
Effective rates	0/60 (0%)	0/44 (0%)	3/67 (4%)	4/38 (11%)
Terminal rates	0/60 (0%)	0/41 (0%)	3/50 (6%)	0/0
First incidence (days)			419 (T)	308
Life table tests	P<0.001	-[e]	P=0.092	P<0.001
Logistic regression tests	P=0.002	-[e]	P=0.092	P=0.011
Cochran-Armitage test	P=0.003			
Fisher exact test		-[e]	P=0.144	P=0.020
All Organs: Benign Tumors				
Overall rates	28/60 (47%)	34/45 (76%)	66/75 (88%)	47/60 (78%)
Effective rates	28/60 (47%)	34/45 (76%)	66/73 (90%)	47/57 (82%)
Terminal rates	28/60 (47%)	33/41 (80%)	48/50 (96%)	0/0
First incidence (days)	419 (T)	405	307	226
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
All Organs: Malignant Tumors				
Overall rates	1/60 (2%)	6/45 (13%)	48/75 (64%)	50/60 (83%)
Effective rates	1/60 (2%)	6/45 (13%)	48/74 (65%)	50/60 (83%)
Terminal rates	1/60 (2%)	2/41 (5%)	29/50 (58%)	0/0
First incidence (days)	419 (T)	250	229	209
Life table tests	P<0.001	P=0.022	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.369	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.023	P<0.001	P<0.001

TABLE A3
Statistical Analysis of Primary Tumors in Male Rats in the 14-Month Drinking Water Study of 3,3'-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
All Organs: Benign and Malignant Tumors				
Overall rates	28/60 (47%)	38/45 (84%)	73/75 (97%)	58/60 (97%)
Effective rates	28/60 (47%)	38/45 (84%)	73/74 (99%)	58/60 (97%)
Terminal rates	28/60 (47%)	34/41 (83%)	50/50 (100%)	0/0
First incidence (days)	419 (T)	250	229	209
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=0.057
Cochran-Armitage test	P<0.001			
Fisher exact test		P<0.001	P<0.001	P<0.001

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined at site.
[b] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the 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 non-fatal. The Cochran-Armitage and Fisher exact tests compare directly the effective tumor rates. For all tests, a negative trend, or a lower incidence in a dose group is indicated by N.
[e] No tumors in dosed group or control group; statistical test not performed.

TABLE A4a
Historical Incidence of Tumors of the Large Intestine in Male F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls	
	Adenocarcinoma	Adenomatous Polyp or Adenocarcinoma[b]
Historical Incidence at Hazleton Laboratories America, Inc.		
Decabromodiphenyl oxide	0/47	
Chlorendic acid	0/49	
Total	0/96 (0%)	
Overall Historical Incidence		
Total	2/1,541 (0.1%)	2/1,541 (0.1%)
Standard deviation	0.4%	0.4%
Range	0%–2%	0%-2%

[a] Data as of 22 November 1989, for studies of at least 104 weeks
[b] No reported incidence for this tumor combination at the study laboratory.

TABLE A4b
Historical Incidence of Tumors of the Small Intestine in Male F344/N Rats Receiving No Treatment[a]

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

[a] Data as of 22 November 1989, for studies of at least 104 weeks
[b] No reported incidence for this tumor combination at the study laboratory.
[c] Includes one (1) carcinoma NOS.

TABLE A4c
Historical Incidence of Liver Tumors in Male F344/N Rats Receiving No Treatment[a]

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

[a] Data as of 22 November 1989, for studies of at least 104 weeks

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

Study	Incidence in Controls	
	Squamous Cell Papilloma	Squamous Cell Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.		
Decabromodiphenyl oxide	0/50	0/50
Chlorendic acid	0/50	0/50
Total	0/100 (0%)	0/100 (0%)
Overall Historical Incidence		
Total	3/1,596 (0.2%)	4/1,596 (0.3%)
Standard deviation	0.5%	0.6%
Range	0%–2%	0%–2%

[a] Data as of 22 November 1989, for studies of at least 104 weeks

TABLE A4e
Historical Incidence of Preputial Gland Tumors in Male F344/N Rats Receiving No Treatment[a]

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

[a] Data as of 22 November 1989, for studies of at least 104 weeks

TABLE A4f
Historical Incidence of Integumentary System Basal Cell Tumors in Male F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls		
	Basal Cell Tumor	Basal Cell Carcinoma	Basal Cell Tumor or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.			
Decabromodiphenyl oxide	0/50	1/50	1/50
Chlorendic acid	0/50	1/50	1/50
Total	0/100 (0%)	2/100 (2%)	2/100 (2%)
Overall Historical Incidence			
Total	11/1,596 (0.7%)	10/1,596 (0.6%)	21/1,596 (1.3%)
Standard deviation	1.4%	1.0%	1.9%
Range	0%–6%	0%–4%	0%–8%

[a] Data as of 22 November 1989, for studies of at least 104 weeks

TABLE A4g
Historical Incidence of Integumentary System Keratoacanthomas in Male F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls
Historical Incidence at Hazleton Laboratories America, Inc.	
Decabromodiphenyl oxide	2/50
Chlorendic acid	4/50
Total	6/100 (6%)
Overall Historical Incidence	
Total	39/1,596 (2.4%)
Standard deviation	3.6%
Range	0%–14%

[a] Data as of 22 November, 1989, for studies of at least 104 weeks

TABLE A4h
Historical Incidence of Integumentary System Squamous Cell Tumors in Male F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.			
Decabromodiphenyl oxide	1/50	1/50	2/50
Chlorendic acid	0/50	1/50	1/50
Total	1/100 (1%)	2/100 (2%)	3/100 (3%)
Overall Historical Incidence			
Total	20/1,596 (1.3%)[b]	9/1,596 (0.6%)	29/1,596 (1.8%)
Standard deviation	1.5%	0.9%	1.6%
Range	0%–4%	0%–2%	0%–4%

[a] Data as of 22 November, 1989, for studies of at least 104 weeks
[b] One (1) papilloma NOS is included in the incidence data.

TABLE A4i
Historical Incidence of Sebaceous Gland Tumors in Male F344/N Rats Receiving No Treatment[a]

	Incidence in Controls
Overall Historical Incidence	
Total	4/1596 (0.3%)
Standard deviation	0.6%
Range	0%–2%

[a] Data as of 22 November, 1989, for studies of at least 104 weeks

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

Study	Incidence in Controls		
	Alveolar/Bronchiolar Adenoma	Alveolar/Bronchiolar Carcinoma	Alveolar/Bronchiolar Adenoma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.			
Decabromodiphenyl oxide	1/50	0/50	1/50
Chlorendic acid	0/50	0/50	0/50
Total	1/100 (1%)	0/100 (0%)	1/100 (1%)
Overall Historical Incidence			
Total	26/1,596 (1.6%)	20/1,596 (1.3%)	44/1,593 (2.8%)
Standard deviation	1.8%	1.8%	2.3%
Range	0%–6%	0%–6%	0%–8%

[a] Data as of 22 November, 1989, for studies of at least 104 weeks

TABLE A4k
Historical Incidence of Zymbal's Gland Tumors in Male F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.			
Decabromodiphenyl oxide	0/50	0/50	0/50
Chlorendic acid	0/50	1/50	1/50
Total	0/100 (0%)	1/100 (1%)	1/100 (1%)
Overall Historical Incidence			
Total	1/1,596 (0.1%)[b]	18/1,596 (1.1%)	19/1,596 (1.2%)
Standard deviation	0.3%	1.8%	1.9%
Range	0%–2%	0%–8%	0%–8%

[a] Data as of 22 November, 1989, for studies of at least 104 weeks
[b] Diagnosed as papillary adenoma

TABLE A4l
Historical Incidence of Mesotheliomas and Malignant Mesotheliomas in Male F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls	
	All Mesotheliomas[b]	Malignant Mesothelioma
Historical Incidence at Hazleton Laboratories America, Inc.		
Decabromodiphenyl oxide	1/50	1/50
Chlorendic acid	2/50	1/50
Total	3/100 (3%)	2/100 (2%)
Overall Historical Incidence		
Total	47/1,596 (2.9%)	11/1,596 (0.7%)
Standard deviation	2.6%	0.9%
Range	0%–10%	0%–2%

[a] Data as of 22 November 1989, for studies of at least 104 weeks
[b] Included are mesothelioma benign, malignant, and NOS

TABLE A4m
Historical Incidence of Brain Tumors in Male F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls	
	Glioma[b]	Astrocytoma
Historical Incidence at Hazleton Laboratories America, Inc.		
Decabromodiphenyl oxide		2/50
Chlorendic acid		0/50
Total		2/100 (2%)
Overall Historical Incidence		
Total	3/1,590 (0.2%)	10/1,590 (0.6%)
Standard deviation	0.5%	1.1%
Range	0%–2%	0%–4%

[a] Data as of 22 November 1989, for studies of at least 104 weeks
[b] No reported incidence for this tumor morphology at study laboratory.

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Month Drinking Water Study of 3,3'-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
Scheduled sacrifice	10			10
Early deaths				
Moribund		2	19	45
Died last week of study		2	5	15
Accident			1	
Survivors				
Terminal sacrifice	60	41	49	
Dead			1	
Animals examined microscopically	60	45	75	60
Alimentary System				
Intestine large, cecum	(60)	(45)	(75)	(58)
Hemorrhage			1 (1%)	
Inflammation, suppurative				1 (2%)
Parasite metazoan	2 (3%)	2 (4%)	2 (3%)	
Intestine large, colon	(60)	(45)	(75)	(57)
Parasite metazoan	8 (13%)	6 (13%)	8 (11%)	9 (16%)
Artery, inflammation, necrotizing				1 (2%)
Intestine large, rectum	(60)	(44)	(75)	(60)
Parasite metazoan	8 (13%)	8 (18%)	5 (7%)	5 (8%)
Intestine small, ileum	(60)	(44)	(75)	(54)
Hemorrhage				1 (2%)
Hyperplasia, lymphoid				1 (2%)
Liver	(60)	(45)	(75)	(60)
Angiectasis				3 (5%)
Basophilic focus	1 (2%)	31 (69%)	54 (72%)	27 (45%)
Congestion			1 (1%)	
Degeneration, cystic		24 (53%)	67 (89%)	51 (85%)
Eosinophilic focus			57 (76%)	53 (88%)
Fatty change, diffuse			1 (1%)	
Fatty change, focal	1 (2%)	1 (2%)		1 (2%)
Fatty change, multifocal		1 (2%)		6 (10%)
Hematocyst				1 (2%)
Hematopoietic cell proliferation		2 (4%)	27 (36%)	15 (25%)
Hemorrhage, focal	1 (2%)			
Hepatodiaphragmatic nodule	3 (5%)	1 (2%)	3 (4%)	6 (10%)
Infarct	1 (2%)		3 (4%)	
Inflammation, acute, focal	3 (5%)			
Inflammation, acute, multifocal	1 (2%)			1 (2%)
Inflammation, granulomatous, multifocal	1 (2%)	5 (11%)	3 (4%)	2 (3%)
Mixed cell focus		37 (82%)	54 (72%)	30 (50%)
Necrosis, coagulative, focal				1 (2%)
Necrosis, coagulative, multifocal	1 (2%)			2 (3%)
Necrosis, focal	2 (3%)	4 (9%)	9 (12%)	1 (2%)
Necrosis, multifocal			1 (1%)	1 (2%)
Bile duct, hyperplasia	39 (65%)	3 (7%)	2 (3%)	1 (2%)
Bile duct, hyperplasia, adenomatous, focal				1 (2%)
Biliary tract, inflammation, subacute			1 (1%)	
Caudate lobe, infarct	1 (2%)		1 (1%)	
Caudate lobe, regeneration				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Alimentary System (continued)				
Caudate lobe, periportal, fibrosis				1 (2%)
Centrilobular, necrosis, coagulative		1 (2%)	1 (1%)	5 (8%)
Serosa, cyst	1 (2%)			
Serosa, fibrosis, focal	1 (2%)			
Serosa, pigmentation, hemosiderin, focal	1 (2%)			
Mesentery	(2)	(6)	(6)	(7)
Hemorrhage	1 (50%)			
Inflammation, granulomatous				1 (14%)
Artery, inflammation, chronic		1 (17%)		
Artery, mineralization				5 (71%)
Fat, necrosis, focal	2 (100%)	5 (83%)	6 (100%)	1 (14%)
Pancreas	(60)	(44)	(75)	(57)
Inflammation, chronic	1 (2%)		1 (1%)	
Acinus, atrophy	9 (15%)	4 (9%)	7 (9%)	
Artery, mineralization				1 (2%)
Salivary glands	(60)	(45)	(74)	(60)
Inflammation, chronic active			1 (1%)	
Necrosis				1 (2%)
Acinus, atrophy			1 (1%)	2 (3%)
Stomach, forestomach	(60)	(45)	(75)	(59)
Erosion, focal			1 (1%)	
Hyperplasia			1 (1%)	
Ulcer			2 (3%)	1 (2%)
Stomach, glandular	(60)	(45)	(75)	(59)
Degeneration, cystic	22 (37%)		4 (5%)	15 (25%)
Ectopic tissue			1 (1%)	
Erosion				1 (2%)
Mineralization				8 (14%)
Ulcer				1 (2%)
Ulcer, multifocal		1 (2%)		
Tongue			(1)	(3)
Hyperplasia, focal				1 (33%)
Tooth	(1)			
Dysplasia		1 (100%)		
Cardiovascular System				
Heart	(60)	(45)	(75)	(59)
Cardiomyopathy	17 (28%)	19 (42%)	35 (47%)	31 (53%)
Dilatation				1 (2%)
Inflammation, acute				1 (2%)
Mineralization				6 (10%)
Atrium, dilatation			1 (1%)	
Atrium, thrombus			2 (3%)	
Mitral valve, inflammation, chronic				1 (2%)
Mitral valve, thrombus				2 (3%)
Myocardium, inflammation, acute				1 (2%)
Myocardium, inflammation, chronic active				1 (2%)
Myocardium, necrosis, focal	1 (2%)			

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Endocrine System				
Adrenal gland, cortex	(60)	(44)	(75)	(59)
Angiectasis				1 (2%)
Hematopoietic cell proliferation			1 (1%)	
Hyperplasia, focal			1 (1%)	
Vacuolization cytoplasmic, focal			5 (7%)	5 (8%)
Adrenal gland, medulla	(60)	(45)	(75)	(59)
Hyperplasia, focal			1 (1%)	
Bilateral, hyperplasia, focal				1 (2%)
Parathyroid gland	(55)	(45)	(71)	(58)
Hyperplasia				11 (19%)
Pituitary gland	(60)	(45)	(75)	(60)
Angiectasis			1 (1%)	
Cyst	2 (3%)	2 (4%)	1 (1%)	1 (2%)
Pars distalis, hyperplasia, focal	2 (3%)		3 (4%)	1 (2%)
Thyroid gland	(60)	(44)	(75)	(60)
Ultimobranchial cyst	3 (5%)		1 (1%)	7 (12%)
C-cell, hyperplasia	1 (2%)		1 (1%)	
Follicle, cyst			1 (1%)	
Follicular cell, hyperplasia, focal			1 (1%)	
General Body System				
Tissue NOS			(1)	(4)
Inflammation, chronic			1 (100%)	
Mineralization				3 (75%)
Genital System				
Epididymis	(60)	(45)	(75)	(60)
Inflammation, chronic			1 (1%)	
Inflammation, suppurative				1 (2%)
Arteriole, inflammation, chronic			1 (1%)	
Preputial gland	(60)	(45)	(75)	(60)
Atrophy			1 (1%)	1 (2%)
Hyperplasia, focal			1 (1%)	3 (5%)
Hyperplasia, squamous, focal				2 (3%)
Inflammation, chronic active	7 (12%)	2 (4%)	9 (12%)	8 (13%)
Duct, ectasia	2 (3%)	2 (4%)	7 (9%)	13 (22%)
Prostate	(60)	(45)	(75)	(60)
Hyperplasia, focal	1 (2%)	2 (4%)		3 (5%)
Hyperplasia, multifocal			1 (1%)	
Inflammation, acute			1 (1%)	
Inflammation, chronic active	35 (58%)	28 (62%)	32 (43%)	15 (25%)
Inflammation, subacute			1 (1%)	
Inflammation, suppurative		1 (2%)		2 (3%)
Vacuolization cytoplasmic, focal		1 (2%)		
Seminal vesicle	(60)	(44)	(75)	(58)
Atrophy		1 (2%)	8 (11%)	5 (9%)
Hyperplasia, focal				2 (3%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Genital System (continued)				
Testes	(60)	(45)	(75)	(60)
Hemorrhage			1 (1%)	1 (2%)
Hypospermia	9 (15%)	3 (7%)	5 (7%)	6 (10%)
Mineralization	3 (5%)			1 (2%)
Interstitial cell, hyperplasia	44 (73%)	35 (78%)	28 (37%)	4 (7%)
Hematopoietic System				
Bone marrow	(60)	(44)	(75)	(60)
Atrophy				8 (13%)
Hemorrhage				8 (13%)
Hyperplasia			(1%)	3 (5%)
Proliferation connective tissue				1 (2%)
Lymph node	(60)	(45)	(75)	(60)
Bronchial, hemorrhage	1 (2%)			
Deep cervical, hyperplasia, plasma cell			1 (1%)	
Mediastinal, hemorrhage	5 (8%)	6 (13%)	9 (12%)	7 (12%)
Mediastinal, hyperplasia, lymphoid	2 (3%)	1 (2%)		
Mediastinal, hyperplasia, plasma cell		1 (2%)	1 (1%)	
Mediastinal, hyperplasia, RE cell			1 (1%)	
Mediastinal, infiltration cellular, polymorphonuclear			1	(2%)
Mediastinal, pigmentation, hemosiderin	2 (3%)			1 (2%)
Mediastinal, sinus, infiltration cellular, histiocytic			1 (1%)	
Pancreatic, hemorrhage	1 (2%)			
Lymph node, mandibular	(60)	(45)	(74)	(60)
Hemorrhage			2 (3%)	1 (2%)
Hyperplasia, lymphoid				3 (5%)
Hyperplasia, plasma cell	2 (3%)	5 (11%)	18 (24%)	16 (27%)
Sinus, ectasia		2 (4%)	3 (4%)	1 (2%)
Lymph node, mesenteric	(60)	(44)	(75)	(56)
Atrophy				1 (2%)
Hemorrhage	1 (2%)		2 (3%)	1 (2%)
Hyperplasia, RE cell	1 (2%)			
Infiltration cellular, polymorphonuclear				1 (2%)
Sinus, ectasia			1 (1%)	1 (2%)
Spleen	(60)	(45)	(75)	(55)
Congestion			1 (1%)	3 (5%)
Depletion lymphoid		1 (2%)		3 (5%)
Fibrosis	1 (2%)		1 (1%)	1 (2%)
Hematopoietic cell proliferation		1 (2%)	22 (29%)	11 (20%)
Hyperplasia, RE cell, focal				1 (2%)
Necrosis				2 (4%)
Necrosis, multifocal				1 (2%)
Thrombus			1 (1%)	
Capsule, fibrosis, focal	1 (2%)			
Capsule, hematopoietic cell proliferation				1 (2%)
Red pulp, necrosis				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Hematopoietic System (continued)				
Thymus	(47)	(40)	(50)	(48)
Congestion				1 (2%)
Cyst				1 (2%)
Depletion lymphoid				3 (6%)
Ectopic parathyroid gland				1 (2%)
Hemorrhage			1 (2%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)			
Epithelial cell, hyperplasia	9 (19%)	9 (23%)	4 (8%)	1 (2%)
Integumentary System				
Skin	(60)	(44)	(75)	(60)
Acanthosis			1 (1%)	1 (2%)
Cyst epithelial inclusion			1 (1%)	
Erosion, focal				1 (2%)
Fibrosis, focal				2 (3%)
Fibrosis, multifocal				1 (2%)
Inflammation, chronic active		1 (2%)	1 (1%)	
Ulcer				1 (2%)
Hair follicle, hyperplasia, basal cell, focal			4 (5%)	1 (2%)
Hair follicle, hyperplasia, basal cell, multifocal			1 (1%)	1 (2%)
Sebaceous gland, hyperplasia, focal			6 (8%)	1 (2%)
Musculoskeletal System				
Bone			(5)	
Cranium, fibrous osteodystrophy				5 (100%)
Sternum, fibrous osteodystrophy				3 (60%)
Sternum, osteopetrosis				2 (40%)
Nervous System				
Brain	(60)	(45)	(75)	(60)
Brain stem, inflammation, acute				1 (2%)
Cerebellum, necrosis				1 (2%)
Cerebrum, hemorrhage				2 (3%)
Cerebrum, hemorrhage, multifocal			1 (1%)	
Cerebrum, necrosis, focal			1 (1%)	1 (2%)
Thalamus, cyst			1 (1%)	
Thalamus, hemorrhage				1 (2%)
Respiratory System				
Lung	(60)	(45)	(75)	(60)
Congestion			2 (3%)	1 (2%)
Embolus			1 (1%)	
Foreign body			1 (1%)	
Granuloma				1 (2%)
Hemorrhage				1 (2%)
Infiltration cellular, histiocytic	9 (15%)	3 (7%)	13 (17%)	13 (22%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Respiratory System				
Inflammation, granulomatous, focal	1 (2%)			
Mineralization				4 (7%)
Necrosis, focal				1 (2%)
Alveolar epithelium, hyperplasia, focal	5 (8%)	11 (24%)	19 (25%)	8 (13%)
Alveolar epithelium, hyperplasia, multifocal		3 (7%)	12 (16%)	9 (15%)
Capillary, thrombus				1 (2%)
Interstitium, inflammation			3 (4%)	4 (7%)
Nose	(60)	(45)	(75)	(60)
Foreign body	2 (3%)	2 (4%)	1 (1%)	
Fungus		1 (2%)	1 (1%)	
Hemorrhage	5 (8%)	5 (11%)	1 (1%)	
Inflammation, acute		1 (2%)		
Inflammation, suppurative	2 (3%)	4 (9%)	3 (4%)	1 (2%)
Trachea	(60)	(45)	(75)	(60)
Inflammation, suppurative			1 (1%)	
Special Senses System				
Eye	(1)			(4)
Cataract	1 (100%)			1 (25%)
Cornea, inflammation, chronic active				1 (25%)
Cornea, inflammation, suppurative				2 (50%)
Retina, degeneration	1 (100%)			
Zymbal's gland	(59)	(45)	(75)	(59)
Cyst			1 (1%)	
Ectasia			1 (1%)	5 (8%)
Hyperplasia, glandular, focal			1 (1%)	1 (2%)
Hyperplasia, squamous, focal				7 (12%)
Hypertrophy				1 (2%)
Inflammation, acute			1 (1%)	
Inflammation, chronic active				2 (3%)
Inflammation, granulomatous			1 (1%)	
Bilateral, hyperplasia, glandular, focal			1 (1%)	
Bilateral, hyperplasia, squamous, focal				3 (5%)
Urinary System				
Kidney	(60)	(45)	(75)	(59)
Cyst		1 (2%)	1 (1%)	1 (2%)
Degeneration, hyaline			1 (1%)	
Hydronephrosis	1 (2%)	1 (2%)	1 (1%)	1 (2%)
Inflammation, acute				2 (3%)
Inflammation, suppurative				1 (2%)
Nephropathy	60 (100%)	43 (96%)	74 (99%)	59 (100%)
Renal tubule, hyperplasia, focal				1 (2%)
Renal tubule, necrosis				1 (2%)
Transitional epithelium, hyperplasia, multifocal				1 (2%)
Urinary bladder	(60)	(45)	(75)	(59)
Dilatation				1 (2%)
Infiltration cellular, lymphocytic	1 (2%)	1 (2%)		
Lumen, hemorrhage	1 (2%)			

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 14-MONTH DRINKING WATER STUDY OF 3,3′-DIMETHYLBENZIDINE DIHYDROCHLORIDE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
Scheduled sacrifice	10			10
Early deaths				
Moribund	1	5	37	45
Dead		1	6	5
Survivors				
Terminal sacrifice	59	39	32	10
Animals examined microscopically	60	45	75	60
Alimentary System				
Intestine large, colon	(60)	(45)	(75)	(60)
Adenocarcinoma				1 (2%)
Leukemia mononuclear			1 (1%)	
Polyp adenomatous		1 (2%)	4 (5%)	2 (3%)
Intestine large, rectum	(60)	(45)	(74)	(60)
Adenocarcinoma			1 (1%)	
Polyp adenomatous			2 (3%)	2 (3%)
Intestine small, duodenum	(60)	(45)	(74)	(59)
Adenocarcinoma				1 (2%)
Intestine small, ileum	(60)	(45)	(75)	(59)
Adenocarcinoma, mucinous				1 (2%)
Polyp adenomatous			1 (1%)	
Intestine small, jejunum	(60)	(45)	(74)	(59)
Adenocarcinoma			1 (1%)	
Adenocarcinoma, cystic, mucinous			1 (1%)	3 (5%)
Leiomyoma		1 (2%)		
Polyp adenomatous		1 (2%)		
Liver	(60)	(45)	(74)	(60)
Hepatocellular carcinoma			1 (1%)	1 (2%)
Hepatocholangiocarcinoma			1 (1%)	
Leukemia mononuclear	1 (2%)	3 (7%)	5 (7%)	4 (7%)
Neoplastic nodule			5 (7%)	3 (5%)
Neoplastic nodule, multiple			2 (3%)	
Pharynx		(1)	(8)	(7)
Papilloma squamous		1 (100%)	5 (63%)	4 (57%)
Squamous cell carcinoma			2 (25%)	3 (43%)
Squamous cell carcinoma, metastatic, Zymbal's gland				
Tongue		(2)	(3)	(10)
Papilloma squamous		2 (100%)	2 (67%)	7 (70%)
Squamous cell carcinoma		1 (50%)		1 (10%)
Cardiovascular System				
Heart	(60)	(45)	(74)	(60)
Epicardium, leukemia mononuclear	1 (2%)			
Endocrine System				
Adrenal gland, cortex	(60)	(45)	(74)	(60)
Leukemia mononuclear			2 (3%)	1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Endocrine System (continued)				
Adrenal gland, medulla	(60)	(45)	(74)	(60)
Leukemia mononuclear			1 (1%)	1 (2%)
Pituitary gland	(58)	(45)	(75)	(60)
Carcinoma, metastatic, Zymbal's gland			1 (1%)	
Pars distalis, adenoma	3 (5%)	1 (2%)	4 (5%)	
Thyroid gland	(60)	(45)	(74)	(60)
C-cell, adenoma	1 (2%)	1 (2%)	2 (3%)	1 (2%)
Follicular cell, adenoma			1 (1%)	1 (2%)
General Body System				
Tissue NOS			(6)	(3)
Carcinoma, metastatic, Zymbal's gland			2 (33%)	2 (67%)
Sarcoma				1 (33%)
Genital System				
Clitoral gland	(60)	(45)	(75)	(59)
Adenoma		8 (18%)	26 (35%)	11 (19%)
Carcinoma		5 (11%)	10 (13%)	16 (27%)
Sarcoma			1 (1%)	
Bilateral, adenoma		1 (2%)	6 (8%)	6 (10%)
Bilateral, carcinoma			1 (1%)	2 (3%)
Ovary	(60)	(44)	(75)	(60)
Choriocarcinoma, metastatic, uterus			1 (1%)	
Granulosa cell tumor malignant				1 (2%)
Granulosa-theca tumor benign		1 (2%)		
Uterus	(60)	(44)	(75)	(60)
Choriocarcinoma			1 (1%)	
Polyp stromal	11 (18%)	9 (20%)	2 (3%)	1 (2%)
Sarcoma stromal		1 (2%)	1 (1%)	
Cervix, polyp stromal			1 (1%)	
Hematopoietic System				
Lymph node	(60)	(45)	(75)	(60)
Carcinoma, metastatic, Zymbal's gland			1 (1%)	
Deep cervical, leukemia mononuclear	1 (2%)			
Mediastinal, leukemia mononuclear			1 (1%)	
Mediastinal, lymphoma malignant		1 (2%)		
Pancreatic, leukemia mononuclear	1 (2%)		1 (1%)	
Renal, leukemia mononuclear	1 (2%)			
Lymph node, mandibular	(60)	(43)	(72)	(60)
Basal cell carcinoma, metastatic, skin			1 (1%)	
Leukemia mononuclear	1 (2%)	1 (2%)	1 (1%)	2 (3%)
Lymph node, mesenteric	(60)	(45)	(73)	(60)
Leukemia mononuclear	1 (2%)	1 (2%)	2 (3%)	3 (5%)
Spleen	(60)	(45)	(74)	(60)
Leukemia mononuclear	1 (2%)	3 (7%)	5 (7%)	3 (5%)
Thymus	(54)	(32)	(67)	(45)
Leukemia mononuclear	1 (2%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Integumentary System				
Mammary gland	(56)	(42)	(73)	(54)
Adenocarcinoma		1 (2%)	3 (4%)	5 (9%)
Adenocarcinoma, multiple				1 (2%)
Fibroadenoma	2 (4%)	1 (2%)	3 (4%)	
Fibroadenoma, multiple			1 (1%)	
Skin	(60)	(45)	(75)	(59)
Basal cell adenoma		3 (7%)	5 (7%)	3 (5%)
Basal cell adenoma, multiple				2 (3%)
Basal cell carcinoma			5 (7%)	4 (7%)
Keratoacanthoma				1 (2%)
Papilloma squamous		1 (2%)	5 (7%)	5 (8%)
Papilloma squamous, multiple			1 (1%)	
Squamous cell carcinoma		2 (4%)	3 (4%)	7 (12%)
Squamous cell carcinoma, multiple			1 (1%)	
Sebaceous gland, adenoma				1 (2%)
Sebaceous gland, carcinoma			1 (1%)	
Subcutaneous tissue, leukemia mononuclear	1 (2%)			
Musculoskeletal System				
Skeletal muscle			(1)	
Sarcoma			1 (100%)	
Nervous System				
Brain	(60)	(45)	(75)	(60)
Astrocytoma malignant		1 (2%)	1 (1%)	
Carcinoma, metastatic, Zymbal's gland			2 (3%)	3 (5%)
Glioma malignant			1 (1%)	1 (2%)
Meningioma malignant		1 (2%)		
Respiratory System				
Lung	(60)	(45)	(74)	(60)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	3 (4%)	3 (5%)
Alveolar/bronchiolar carcinoma				1 (2%)
Basal cell carcinoma, metastatic, skin			1 (1%)	
Carcinoma, metastatic, clitoral gland			1 (1%)	2 (3%)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Carcinoma, metastatic, Zymbal's gland			2 (3%)	1 (2%)
Leukemia mononuclear	1 (2%)		4 (5%)	1 (2%)
Squamous cell carcinoma, metastatic, pharynx				1 (2%)
Mediastinum, sarcoma, metastatic, skeletal muscle			1 (1%)	
Nose	(60)	(44)	(75)	(60)
Carcinoma, metastatic, Zymbal's gland			1 (1%)	
Special Senses System				
Ear			(1)	
Canal, papilloma squamous				1 (100%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Special Senses System (continued)				
Eye	(1)	(4)	(4)	
Conjunctiva, squamous cell carcinoma				1 (25%)
Zymbal's gland	(57)	(44)	(73)	(60)
Adenoma		4 (9%)	9 (12%)	12 (20%)
Carcinoma		2 (5%)	20 (27%)	34 (57%)
Bilateral, adenoma			2 (3%)	
Bilateral, carcinoma			2 (3%)	1 (2%)
Urinary System				
Kidney	(60)	(45)	(74)	(60)
Leukemia mononuclear	1 (2%)			1 (2%)
Renal tubule, adenoma				1 (2%)
Urinary bladder	(60)	(45)	(75)	(60)
Leukemia mononuclear			1 (1%)	
Sarcoma stromal, metastatic, uterus			1 (1%)	
Systemic Lesions				
Multiple organs	(60)*	(45)*	(75)*	(60)*
Leukemia mononuclear	1 (2%)	3 (7%)	6 (8%)	4 (7%)
Lymphoma malignant		1 (2%)		
Tumor Summary				
Total animals with primary neoplasms**	18	33	71	58
Total primary neoplasms	19	55	157	157
Total animals with benign neoplasms	17	24	54	41
Total benign neoplasms	18	37	92	67
Total animals with malignant neoplasms	1	14	49	56
Total malignant neoplasms	1	18	65	90
Total animals with secondary neoplasms***			11	9
Total secondary neoplasms			16	10
Total animals with malignant neoplasms, Uncertain primary site				1

* Number of animals with any tissue examined microscopically
** Primary tumors: all tumors except metastatic tumors
*** Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm

Number of Days on Study	3 9 0	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2
Carcass ID Number	2 0 5	1 7 1	1 7 2	1 7 3	1 7 4	1 7 5	1 8 1	1 8 2	1 8 3	1 8 4	1 8 5	1 9 1	1 9 2	1 9 3	1 9 4	1 9 5	2 0 1	2 0 2	2 0 3	2 0 4	2 1 1	2 1 2	2 1 3	2 1 4	2 1 5
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																								
Mesentery	+																								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Epicardium, leukemia mononuclear	X																								
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma								X			X		X												
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma									X																
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal		X											X			X			X					X	

+: Tissue examined
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 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm (continued)

Number of Days on Study	4 2 2	4 2 2	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6
Carcass ID Number	2 2 1	2 2 2	2 2 3	2 2 4	2 2 5	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 4 1	2 4 2	2 4 3	2 4 4	2 4 5	2 5 1	2 5 2	2 5 3	2 5 4	2 5 5	2 6 1	2 6 2	2 6 3	2 6 4	2 6 5
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																									
Mesentery										+		+													
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Epicardium, leukemia mononuclear																									
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Pituitary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal				X							X				X					X			X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm (continued)

Number of Days on Study	4 2 6	4 2 6	4 2 7	4 2 7	4 2 7	4 2 7	4 2 7	4 2 7	4 2 7	4 2 7	
Carcass ID Number	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	+	+	+	+	+	+	+	+	+	+	60
Intestine large	+	+	+	+	+	+	+	+	+	+	60
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	60
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	60
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	60
Intestine small	+	+	+	+	+	+	+	+	+	+	60
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	60
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	60
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	60
Liver	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Mesentery											3
Pancreas	+	+	+	+	+	+	+	+	+	+	60
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Stomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	60
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	60
Epicardium, leukemia mononuclear											1
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	60
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	60
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	53
Pituitary gland	+	+	+	M	+	+	+	+	+	+	58
Pars distalis, adenoma											3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	60
C-cell, adenoma											1
General Body System											
None											
Genital System											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	60
Ovary	+	+	+	+	+	+	+	+	+	+	60
Uterus	+	+	+	+	+	+	+	+	+	+	60
Polyp stromal			X								11

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm (continued)

Number of Days on Study	3 9 0	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2
Carcass ID Number	2 0 5	1 7 1	1 7 2	1 7 3	1 7 4	1 7 5	1 8 1	1 8 2	1 8 3	1 8 4	1 8 5	1 9 1	1 9 2	1 9 3	1 9 4	1 9 5	2 0 1	2 0 2	2 0 3	2 0 4	2 1 1	2 1 2	2 1 3	2 1 4	2 1 5
Hematopoietic System																									
Bone Marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deep cervical, leukemia mononuclear	X																								
Pancreatic, leukemia mononuclear	X																								
Renal, leukemia mononuclear	X																								
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+
Leukemia mononuclear	X																								
Integumentary System																									
Mammary gland	+	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma										X															
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, leukemia mononuclear	X																								
Musculoskeletal System																									
None																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Leukemia mononuclear	X																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm (continued)

Number of Days on Study	4 2 2	4 2 2	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6	4 2 6
Carcass ID Number	2 2 1	2 2 2	2 2 3	2 2 4	2 2 5	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 4 1	2 4 2	2 4 3	2 4 4	2 4 5	2 5 1	2 5 2	2 5 3	2 5 4	2 5 5	2 6 1	2 6 2	2 6 3	2 6 4	2 6 5
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deep cervical, leukemia mononuclear																									
Pancreatic, leukemia mononuclear																									
Renal, leukemia mononuclear																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Fibroadenoma					X																				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, leukemia mononuclear																									
Musculoskeletal System																									
None																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																					X				
Leukemia mononuclear																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 0 ppm (continued)

Number of Days on Study	4 2 6	4 2 6	4 2 7	4 2 7	4 2 7	4 2 7	4 2 7	4 2 7	4 2 7	4 2 7	
Carcass ID Number	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**
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	60
Lymph node	+	+	+	+	+	+	+	+	+	+	60
Deep cervical, leukemia mononuclear											1
Pancreatic, leukemia mononuclear											1
Renal, leukemia mononuclear											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Spleen	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Thymus	+	M	M	+	+	+	+	+	+	+	54
Leukemia mononuclear											1
Integumentary System											
Mammary gland	+	+	+	+	+	+	+	+	+	+	56
Fibroadenoma											2
Skin	+	+	+	+	+	+	+	+	+	+	60
Subcutaneous tissue, leukemia mononuclear											1
Musculoskeletal System											
None											
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	60
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar adenoma											1
Leukemia mononuclear											1
Nose	+	+	+	+	+	+	+	+	+	+	60
Trachea	+	+	+	+	+	+	+	+	+	+	60
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	57
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	60
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm

Number of Days on Study	3 5 7	3 8 4	3 9 1	3 9 1	3 9 1	3 9 3	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2
Carcass ID Number	3 9 5	4 5 5	4 0 5	4 3 5	4 4 5	3 9 4	3 8 1	3 8 2	3 8 3	3 8 4	3 8 5	3 9 1	3 9 2	3 9 3	4 0 1	4 0 2	4 0 3	4 0 4	4 1 1	4 1 2	4 1 3	4 1 4	4 1 5	4 2 2	4 2 3
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									
Polyp adenomatous				X																					
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Mesentery							+																		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx																									
Papilloma squamous																									
Salivary glands	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Papilloma squamous																									
Squamous cell carcinoma																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
General Body																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm (continued)

Number of Days on Study	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 6	4 2 6	4 2 6	4 2 6	
Carcass ID Number	4 2 5	4 3 1	4 3 2	4 3 3	4 4 1	4 4 2	4 4 3	4 4 4	4 5 1	4 5 2	4 5 3	4 5 4	4 6 1	4 6 2	4 6 3	4 6 4	4 2 1	4 2 4	4 3 4	4 6 5	**Total Tissues/ Tumors**
Alimentary System																					
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Polyp adenomatous								X													1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leiomyoma																X					1
Polyp adenomatous																					1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear	X															X		X			3
Mesentery								+					+								3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pharynx																+					1
Papilloma squamous																X					1
Salivary glands	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Tongue										+				+							2
Papilloma squamous										X				X							2
Squamous cell carcinoma										X											1
Cardiovascular System																					
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Endocrine System																					
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pars distalis, adenoma										X											1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
C-cell, adenoma																	X				1
General Body System																					
None																					

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm (continued)

Number of Days on Study	3 5 7	3 8 4	3 9 1	3 9 1	3 9 1	3 9 3	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2
Carcass ID Number	3 9 5	4 5 5	4 0 5	4 3 5	4 4 5	3 9 4	3 8 1	3 8 2	3 8 3	3 8 4	3 8 5	3 9 1	3 9 2	3 9 3	4 0 1	4 0 2	4 0 3	4 0 4	4 1 1	4 1 2	4 1 3	4 1 4	4 1 5	4 2 2	4 2 3
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X	X		X							X										X		
Carcinoma									X								X								
Bilateral, adenoma					X																				
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa-theca tumor benign						X																			
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal				X															X				X		
Sarcoma stromal									X																
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, lymphoma malignant	X																								
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Thymus	+	M	+	M	M	M	+	M	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M
Integumentary System																									
Mammary gland	+	+	+	+	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma								X																	
Papilloma squamous												X													
Squamous cell carcinoma																									
Musculoskeletal System																									
Bone		+																			+				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																X									
Meningioma malignant		X																							

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3'-Dimethylbenzidine Dihydrochloride: 30 ppm (continued)

Number of Days on Study	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 6	4 2 6	4 2 6	4 2 6	
Carcass ID Number	4 2 5	4 3 1	4 3 2	4 3 3	4 4 1	4 4 2	4 4 3	4 4 4	4 5 1	4 5 2	4 5 3	4 5 4	4 6 1	4 6 2	4 6 3	4 6 4	4 2 1	4 2 4	4 3 4	4 6 5	**Total Tissues/ Tumors**	
Genital System																						
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45	
Adenoma							X	X												X	8	
Carcinoma												X					X		X		5	
Bilateral, adenoma																					1	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	44	
Granulosa-theca tumor benign																					1	
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	44	
Polyp stromal						X					X		X	X	X			X			9	
Sarcoma stromal																					1	
Hematopoietic System																						
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45	
Mediastinal, lymphoma malignant																					1	
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43	
Leukemia mononuclear																		X			1	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45	
Leukemia mononuclear																		X			1	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45	
Leukemia mononuclear	X															X		X			3	
Thymus	+	+	+	+	+	M	M	M	+	+	+	+	+	+	M	+	+	+	+	M	32	
Integumentary System																						
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42	
Adenocarcinoma																			X		1	
Fibroadenoma										X											1	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45	
Basal cell adenoma							X			X											3	
Papilloma squamous																					1	
Squamous cell carcinoma					X													X			2	
Musculoskeletal System																						
Bone																					2	
Nervous System																						
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45	
Astrocytoma malignant																					1	
Meningioma malignant																					1	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm (continued)

Number of Days on Study	3 5 7	3 8 4	3 9 1	3 9 1	3 9 1	3 9 3	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2
Carcass ID Number	3 9 5	4 5 5	4 0 5	4 3 5	4 4 5	3 9 4	3 8 1	3 8 2	3 8 3	3 8 4	3 8 5	3 9 1	3 9 2	3 9 3	4 0 1	4 0 2	4 0 3	4 0 4	4 1 1	4 1 2	4 1 3	4 1 4	4 1 5	4 2 2	4 2 3
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																		X							
Nose	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Zymbal's gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X													X	
Carcinoma	X			X																					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Lymphoma malignant	X																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 30 ppm (continued)

Number of Days on Study	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 6	4 2 6	4 2 6	4 2 6	
Carcass ID Number	4 2 5	4 3 1	4 3 2	4 3 3	4 4 1	4 4 2	4 4 3	4 4 4	4 5 1	4 5 2	4 5 3	4 5 4	4 6 1	4 6 2	4 6 3	4 6 4	4 2 1	4 2 4	4 3 4	4 6 5	**Total Tissues/ Tumors**
Respiratory System																					
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Alveolar/bronchiolar adenoma																					1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Special Senses System																					
Eye													+								1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Adenoma							X	X													4
Carcinoma																					2
Urinary System																					
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Systemic Lesions																					
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Leukemia mononuclear	X															X		X			3
Lymphoma malignant																					1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	
Carcass ID Number	6 4 2	6 5 1	6 6 1	6 6 2	6 7 1	6 8 1	6 9 1	6 9 2	7 0 1	7 0 2	7 1 1	7 1 1	7 2 2	7 2 2	7 3 1	7 3 2	7 4 1	7 4 2	7 4 3	7 4 4	7 5 1	7 5 2	7 5 3	7 6 1	7 7 2	7 7 4	**Total Tissues/ Tumors**
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Leukemia mononuclear																											1
Polyp adenomatous													X								X						4
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Adenocarcinoma																											1
Polyp adenomatous																							X				2
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Polyp adenomatous																											1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Adenocarcinoma																											1
Adenocarcinoma, cystic, mucinous																											1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Hepatocellular carcinoma											X																1
Hepatocholangiocarcinoma													X														1
Leukemia mononuclear																			X	X		X					5
Neoplastic nodule								X			X									X							5
Neoplastic nodule, multiple															X						X						2
Mesentery																											1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Pharynx													+		+				+	+				+			8
Papilloma squamous													X							X							5
Squamous cell carcinoma																			X					X			2
Squamous cell carcinoma, metastatic, Zymbal's gland																											1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	72
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Tongue																											3
Papilloma squamous																											2
Tooth																											2
Cardiovascular System																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	1 4 2	1 4 4	2 2 9	2 6 8	3 0 9	3 1 0	3 1 5	3 1 5	3 1 5	3 2 0	3 2 7	3 3 8	3 3 8	3 3 8	3 3 8	3 5 5	3 6 3	3 6 4	3 6 5	3 6 9	3 6 9	3 7 0	3 7 8	3 7 9
Carcass ID Number	6 9 5	6 5 5	7 1 5	7 5 5	6 8 5	6 9 4	6 2 5	6 8 3	6 8 4	7 6 5	7 2 5	6 2 4	6 3 5	7 2 4	7 3 5	7 6 4	7 6 5	7 6 3	7 7 4	7 2 3	7 1 3	7 1 4	6 5 4	6 6 4	6 6 5
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Leukemia mononuclear																	X							
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Leukemia mononuclear																	X							
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland										X														
Pars distalis, adenoma																	X	X						
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																								
Follicular cell, adenoma																						X		
General Body System																								
Tissue NOS								+		+	+							+		+			+	
Carcinoma, metastatic, Zymbal's gland										X	X													
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X	X		X				X								X	X					X
Carcinoma									X					X						X		X	X	
Sarcoma																								
Bilateral, adenoma					X												X							
Bilateral, carcinoma																								
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Choriocarcinoma, metastatic, uterus																								
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Choriocarcinoma																								
Polyp stromal													X											
Sarcoma stromal																								
Cervix, polyp stromal																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 8 8 8 9 9 9 9 9 0 0 0 0 0 0 0 0 0 1 2 2 2 2 2 2 2 3 4 8 0 0 1 1 1 5 5 5 6 6 6 6 6 9 9 1 1 1 1 1 2 2
Carcass ID Number	7 6 7 6 7 6 7 7 6 7 7 6 6 6 6 6 7 6 6 6 6 6 6 6 4 9 6 5 3 6 0 0 4 1 3 4 4 5 7 8 0 3 2 2 2 3 3 3 4 5 3 2 3 4 3 4 5 4 2 3 3 5 2 3 2 3 2 1 2 3 1 3 4 1
Endocrine System	
Adrenal gland	+ +
Adrenal gland, cortex	+ +
Leukemia mononuclear	
Adrenal gland, medulla	+ +
Leukemia mononuclear	
Islets, pancreatic	+ +
Parathyroid gland	+ +
Pituitary gland	+ +
Carcinoma, metastatic, Zymbal's gland	
Pars distalis, adenoma	
Thyroid gland	+ +
C-cell, adenoma	X
Follicular cell, adenoma	
General Body System	
Tissue NOS	
Carcinoma, metastatic, Zymbal's gland	
Genital System	
Clitoral gland	+ +
Adenoma	X X X X X X X X X X X
Carcinoma	X X X X
Sarcoma	X
Bilateral, adenoma	X X X
Bilateral, carcinoma	
Ovary	+ +
Choriocarcinoma, metastatic, uterus	X
Uterus	+ +
Choriocarcinoma	X
Polyp stromal	X
Sarcoma stromal	
Cervix, polyp stromal	X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 6	4 2 6	
Carcass ID Number	6 4 2	6 5 1	6 6 1	6 6 2	6 7 1	6 8 1	6 9 1	6 9 2	7 0 1	7 0 2	7 1 1	7 2 1	7 2 2	7 3 1	7 3 2	7 4 1	7 4 2	7 4 3	7 4 4	7 5 1	7 5 2	7 5 3	7 6 1	7 6 2	7 5 4	**Total Tissues/ Tumors**
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Leukemia mononuclear																					X					2
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Leukemia mononuclear																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Parathyroid gland	+	+	+	+	M	+	+		+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	70
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Carcinoma, metastatic, Zymbal's gland																										1
Pars distalis, adenoma		X											X													4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
C-cell, adenoma								X																		2
Follicular cell, adenoma																										1
General Body System																										
Tissue NOS																										6
Carcinoma, metastatic, Zymbal's gland																										2
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Adenoma				X		X				X	X		X		X	X	X								X	26
Carcinoma				X																				X		10
Sarcoma																										1
Bilateral, adenoma							X																			6
Bilateral, carcinoma																						X				1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Choriocarcinoma, metastatic, uterus																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Choriocarcinoma																										1
Polyp stromal																										2
Sarcoma stromal															X											1
Cervix, polyp stromal																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	1 4 2	1 8 4	2 2 9	2 6 8	3 0 9	3 1 0	3 1 5	3 1 5	3 1 5	3 2 0	3 2 7	3 3 8	3 3 8	3 3 8	3 3 8	3 3 5	3 5 5	3 6 3	3 6 4	3 6 5	3 6 9	3 6 9	3 7 0	3 7 8	3 7 9
Carcass ID Number	6 9 5	6 5 5	7 1 5	7 5 5	6 8 5	6 9 4	6 2 5	6 8 3	6 8 4	7 6 5	7 2 5	6 2 4	6 3 5	7 2 4	7 3 5	7 6 4	7 6 5	7 6 3	7 7 4	7 2 3	7 1 3	7 1 4	7 5 4	6 6 4	6 6 5
Hematopoietic System																									
Blood																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland		X																							
Mediastinal, leukemia mononuclear																		X							
Pancreatic, leukemia mononuclear																		X							
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma, metastatic, skin																									
Leukemia mononuclear																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Leukemia mononuclear																		X							
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Leukemia mononuclear																		X							
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Adenocarcinoma																		X				X			
Fibroadenoma																									
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma												X	X												
Basal cell carcinoma									X																
Papilloma squamous																									
Papilloma squamous, multiple																									
Squamous cell carcinoma																									
Squamous cell carcinoma, multiple																									
Sebaceous gland, carcinoma																									
Musculoskeletal System																									
Bone								+													+				
Skeletal muscle																									
Sarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									
Carcinoma, metastatic, Zymbal's gland										X															
Glioma malignant																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	3 8 3	3 8 4	3 8 8	3 9 0	3 9 0	3 9 1	3 9 1	3 9 1	4 0 5	4 0 5	4 0 5	4 0 6	4 0 6	4 0 6	4 0 6	4 0 6	4 0 9	4 1 9	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 2	4 2 2
Carcass ID Number	7 4 5	6 9 3	7 6 2	6 5 3	7 3 4	6 6 3	7 0 4	7 0 5	6 4 4	7 1 2	7 3 3	6 4 3	6 4 5	6 5 2	6 7 3	6 8 2	7 0 3	6 3 2	6 2 1	6 2 2	6 2 3	6 3 1	6 3 3	6 3 4	6 4 1
Hematopoietic System																									
Blood																									
Bone marrow	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland																									
Mediastinal, leukemia mononuclear																									
Pancreatic, leukemia mononuclear																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Basal cell carcinoma, metastatic, skin												X													
Leukemia mononuclear															X										
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Leukemia mononuclear															X										
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear															X										
Thymus	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Fibroadenoma																X									
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Basal cell carcinoma			X									X				X									X
Papilloma squamous						X	X																	X	
Papilloma squamous, multiple																									
Squamous cell carcinoma																X									
Squamous cell carcinoma, multiple																									
Sebaceous gland, carcinoma																									
Musculoskeletal System																									
Bone																									
Skeletal muscle											+														
Sarcoma											X														
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									
Carcinoma, metastatic, Zymbal's gland	X																								
Glioma malignant																		X							

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 6	4 2 6	
Carcass ID Number	6 4 2	6 5 1	6 6 1	6 6 2	6 7 1	6 8 1	6 9 1	6 9 2	7 0 1	7 0 2	7 1 1	7 2 1	7 2 2	7 3 1	7 3 2	7 4 1	7 4 2	7 4 3	7 4 4	7 5 1	7 5 2	7 5 3	7 6 1	7 7 2	7 5 4	Total Tissues/ Tumors
Hematopoietic System																										
Blood															+											1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	73
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Carcinoma, metastatic, Zymbal's gland																										1
Mediastinal, leukemia mononuclear																										1
Pancreatic, leukemia mononuclear																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	72
Basal cell carcinoma, metastatic, skin																										1
Leukemia mononuclear																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	73
Leukemia mononuclear																										2
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Leukemia mononuclear				X														X			X					5
Thymus	+	+	M	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	M	+	+	+	+	+	+	67
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	73
Adenocarcinoma			X																							3
Fibroadenoma		X													X											3
Fibroadenoma, multiple																									X	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Basal cell adenoma														X						X				X		5
Basal cell carcinoma																										5
Papilloma squamous	X		X																							5
Papilloma squamous, multiple																					X					1
Squamous cell carcinoma		X										X														3
Squamous cell carcinoma, multiple																					X					1
Sebaceous gland, carcinoma																						X				1
Musculoskeletal System																										
Bone																										2
Skeletal muscle																										1
Sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Astrocytoma malignant																							X			1
Carcinoma, metastatic, Zymbal's gland																										2
Glioma malignant																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	1 4 2	1 8 4	2 2 9	2 6 8	3 0 9	3 1 0	3 1 5	3 1 5	3 1 5	3 2 0	3 2 7	3 3 8	3 3 8	3 3 8	3 3 8	3 3 8	3 5 5	3 6 3	3 6 4	3 6 5	3 6 9	3 6 9	3 7 0	3 7 8	3 7 9
Carcass ID Number	6 9 5	6 5 5	7 1 5	7 5 5	6 8 5	6 9 4	6 2 5	6 8 3	6 8 4	7 6 5	7 2 5	6 2 4	6 3 5	7 2 4	7 3 5	7 6 4	7 7 5	6 7 3	7 2 4	7 3 3	7 1 3	7 1 4	6 5 4	6 6 4	6 6 5
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma													X												
Basal cell carcinoma, metastatic, skin																									
Carcinoma, metastatic, clitoral gland																									
Carcinoma, metastatic, Zymbal's gland		X					X																		
Leukemia mononuclear																		X							
Mediastinum, sarcoma, metastatic skeletal muscle																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland										X															
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye										+							+								+
Harderian gland																	+								
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X		X				X	X				X		
Carcinoma		X		X	X		X			X	X	X	X			X	X				X				
Bilateral, adenoma																									
Bilateral, carcinoma																									X
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																									
Sarcoma stromal, metastatic																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																		X							

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 8 8 8 9 9 9 9 9 0 0 0 0 0 0 0 0 0 1 2 2 2 2 2 2 2 3 4 8 0 0 1 1 1 5 5 5 6 6 6 6 6 9 9 1 1 1 1 1 2 2
Carcass ID Number	7 6 7 6 7 6 7 7 6 7 7 6 6 6 6 6 7 6 6 6 6 6 6 6 6 4 9 6 5 3 6 0 0 4 1 3 4 4 5 7 8 0 3 2 2 2 3 3 3 4 5 3 2 3 4 3 4 5 4 2 3 3 5 2 3 2 3 2 1 2 3 1 3 4 1
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	X
Basal cell carcinoma, metastatic, skin	X
Carcinoma, metastatic, clitoral gland	X
Carcinoma, metastatic, Zymbal's gland	
Leukemia mononuclear	X
Mediastinum, sarcoma, metastatic, skeletal muscle	X
Nose	+ +
Carcinoma, metastatic, Zymbal's gland	
Trachea	+ +
Special Senses System	
Eye	
Harderian gland	
Zymbal's gland	+ + + + + + + + + + M + + + + + + + + + + + + + +
Adenoma	X
Carcinoma	X X X X X X X X X
Bilateral, adenoma	
Bilateral, carcinoma	X
Urinary System	
Kidney	+ +
Urinary bladder	+ +
Leukemia mononuclear	X
Sarcoma stromal, metastatic	
Systemic Lesions	
Multiple organs	+ +
Leukemia mononuclear	X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 70 ppm (continued)

Number of Days on Study	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 2	4 2 5	4 2 5	4 2 5	4 2 5	4 2 5	4 2 6	4 2 6	
Carcass ID Number	6 4 2	6 5 1	6 6 1	6 6 2	6 7 1	6 8 1	6 9 1	6 9 2	7 0 1	7 0 2	7 1 1	7 2 1	7 2 2	7 3 1	7 3 2	7 4 1	7 4 2	7 4 3	7 4 4	7 5 1	7 5 2	7 5 3	7 6 1	7 7 2	7 5 4	**Total Tissues/ Tumors**
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Alveolar/bronchiolar adenoma					X																					3
Basal cell carcinoma, metastatic, skin																										1
Carcinoma, metastatic, clitoral gland																										1
Carcinoma, metastatic, Zymbal's gland																										2
Leukemia mononuclear																		X			X					4
Mediastinum, sarcoma, metastatic, skeletal muscle																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Carcinoma, metastatic, Zymbal's gland																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Special Senses System																										
Eye								+																		4
Harderian gland																										1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	73
Adenoma				X												X						X				9
Carcinoma								X																		20
Bilateral, adenoma																			X	X						2
Bilateral, carcinoma																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	74
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Leukemia mononuclear																										1
Sarcoma stromal, metastatic															X											1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	75
Leukemia mononuclear				X														X	X		X					6

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm

Number of Days on Study	1 4 2	2 2 9	2 4 7	2 5 1	2 5 4	2 6 4	2 6 4	2 6 5	2 8 0	2 8 5	2 8 5	2 9 5	2 9 6	2 9 6	3 1 5	3 1 5	3 3 5	3 3 5	3 3 6	3 3 8	3 3 8	3 4 1	4 1	4 3
Carcass ID Number	0 9 3 5	0 9 3 4	1 0 2 5	1 0 2 4	0 9 2 5	0 9 5 5	1 0 0 5	0 9 4 5	0 9 4 4	1 0 1 4	1 0 1 5	0 9 9 5	0 9 6 5	0 9 8 5	0 9 7 4	0 9 7 5	1 0 2 3	1 0 3 5	0 9 6 4	0 9 4 3	0 9 6 3	0 9 8 4	0 9 4 2	0 9 6 2	0 9 5 3
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																								
Polyp adenomatous																								
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																								
Intestine small	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma				X																				
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, mucinous																								
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, cystic, mucinous																								
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																								
Leukemia mononuclear																								
Neoplastic nodule																								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx		+																						+
Papilloma squamous		X																						
Squamous cell carcinoma																								X
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue											+										+			
Papilloma squamous											X													
Squamous cell carcinoma																					X			
Tooth																	+							
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	3 4 3	3 4 3	3 5 5	3 5 5	3 6 4	3 7 0	3 7 0	3 7 7	3 7 8	3 8 3	3 8 3	3 8 4	3 8 4	3 9 0	3 9 1	3 9 1	3 9 1	3 9 1	4 0 5	4 0 5	4 0 5	4 0 6	4 0 6	4 0 6
Carcass ID Number	0 9 5 4	1 0 3 4	0 9 3 2	0 9 3 3	1 0 1 3	1 0 0 4	1 0 4 5	0 9 8 3	1 0 0 3	1 0 1 2	1 0 3 3	1 0 3 2	1 0 4 4	0 9 8 2	0 9 6 1	0 9 7 3	1 0 1 1	1 0 2 2	1 0 4 3	1 0 9 3	1 0 4 1	1 0 4 2	0 9 5 2	0 9 4 4	1 0 2 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																						X			
Polyp adenomatous				X																					
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																				X		X			
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, mucinous																									X
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, cystic, mucinous																	X			X		X			
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																						X			
Leukemia mononuclear					X																X	X			
Neoplastic nodule		X	X																				X		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx						+															+				
Papilloma squamous																					X				
Squamous cell carcinoma						X																			
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue	+						+			+		+									+				
Papilloma squamous							X			X		X									X				
Squamous cell carcinoma																									
Tooth																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X																				
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X																				
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 2	4 2 2	4 2 2	4 2 2	4 2 6	
Carcass ID Number	0 9 4 1	0 9 5 1	0 9 7 1	0 9 7 2	0 9 8 1	0 9 9 1	0 9 9 2	1 0 2 1	1 0 3 1	1 0 0 1	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	60
Intestine large	+	+	+	+	+	+	+	+	+	+	60
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	60
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	60
Adenocarcinoma											1
Polyp adenomatous										X	2
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	60
Polyp adenomatous											2
Intestine small	+	+	+	+	+	+	+	+	+	+	59
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	59
Adenocarcinoma											1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	59
Adenocarcinoma, mucinous											1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	59
Adenocarcinoma, cystic, mucinous											3
Liver	+	+	+	+	+	+	+	+	+	+	60
Hepatocellular carcinoma											1
Leukemia mononuclear		X									4
Neoplastic nodule											3
Pancreas	+	+	+	+	+	+	+	+	+	+	60
Pharynx						+	+			+	7
Papilloma squamous						X				X	4
Squamous cell carcinoma							X				3
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Stomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	60
Tongue	+								+	+	10
Papilloma squamous									X	X	7
Squamous cell carcinoma											1
Tooth											1
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	60
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	60
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	58
Pituitary gland	+	+	+	+	+	+	+	+	+	+	60

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	1 4 2	2 2 9	2 4 7	2 5 1	2 5 4	2 6 4	2 6 4	2 6 5	2 8 0	2 8 5	2 8 5	2 9 5	2 9 6	2 9 6	3 1 5	3 1 5	3 3 5	3 3 5	3 3 6	3 3 8	3 3 8	3 4 1	4 4 1	3 4 3
Carcass ID Number	0 9 3 5	0 9 3 4	1 0 2 5	1 0 2 4	0 9 2 5	0 9 5 5	1 0 0 5	0 9 4 5	0 9 4 4	1 0 1 4	1 0 1 5	0 9 9 5	0 9 6 5	0 9 8 5	0 9 7 4	0 9 7 5	1 0 2 3	1 0 3 5	0 9 6 4	0 9 4 3	0 9 6 3	0 9 8 4	0 9 4 2	0 9 6 2	0 9 5 3
Endocrine System (continued)																								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma														X										
Follicular cell, adenoma																								
General Body System																								
Tissue NOS					+							+												
Carcinoma, metastatic, Zymbal's gland					X							X												
Sarcoma																								
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Adenoma															X	X								
Carcinoma					X									X	X				X		X			
Bilateral, adenoma													X					X				X		
Bilateral, carcinoma																								
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																								
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																								
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																								
Mammary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	M	+
Adenocarcinoma											X													
Adenocarcinoma, multiple																								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+
Basal cell adenoma																								
Basal cell adenoma, multiple																								
Basal cell carcinoma																								
Keratoacanthoma																			X					
Papilloma squamous							X																	
Squamous cell carcinoma																					X			
Sebaceous gland, adenoma																				X				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	3 4 3	3 4 3	3 5 5	3 5 5	3 6 4	3 7 0	3 7 0	3 7 7	3 7 8	3 8 3	3 8 3	3 8 4	3 8 4	3 9 0	3 9 1	3 9 1	3 9 1	3 9 1	3 9 1	4 0 5	4 0 5	4 0 5	4 0 6	4 0 6	4 0 6
Carcass ID Number	0 9 5 4	1 0 3 4	0 9 3 2	0 9 3 3	1 0 1 3	1 0 0 4	1 0 4 5	0 9 8 3	1 0 0 3	1 0 1 2	1 0 3 3	1 0 3 2	1 0 4 4	0 9 8 2	0 9 6 1	0 9 7 3	0 9 1 1	1 0 2 2	1 0 4 3	1 0 9 3	1 0 4 1	1 0 4 2	0 9 5 2	0 9 9 4	1 0 0 2
Endocrine System (continued)																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
Follicular cell, adenoma																									
General Body System																									
Tissue NOS																			+						
Carcinoma, metastatic, Zymbal's gland																									
Sarcoma																			X						
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X	X		X					X									X					
Carcinoma										X					X	X	X	X	X	X		X		X	
Bilateral, adenoma		X																			X				
Bilateral, carcinoma													X												
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor malignant																			X						
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																						X			
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																				X	X				
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X															X	X				
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X															X					
Thymus	+	+	+	M	+	M	+	+	I	+	M	+	M	+	+	M	+	+	M	M	+	M	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma								X				X													
Adenocarcinoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma							X							X											
Basal cell adenoma, multiple																				X					
Basal cell carcinoma	X	X																				X			
Keratoacanthoma																									
Papilloma squamous											X					X							X		
Squamous cell carcinoma												X		X	X										
Sebaceous gland, adenoma																									X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 2	4 2 2	4 2 2	4 2 2	4 2 6	
Carcass ID Number	0 9 4 1	0 9 5 1	0 9 7 1	0 9 7 2	0 9 8 1	0 9 9 1	0 9 9 2	1 0 2 1	1 0 3 1	1 0 0 1	**Total Tissues/ Tumors**
Endocrine System (continued)											
Thyroid gland	+	+	+	+	+	+	+	+	+	+	60
C-cell, adenoma											1
Follicular cell, adenoma								X			1
General Body System											
Tissue NOS											3
Carcinoma, metastatic, Zymbal's gland											2
Sarcoma											1
Genital System											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	59
Adenoma		X		X				X		X	11
Carcinoma		X				X					16
Bilateral, adenoma					X						6
Bilateral, carcinoma									X		2
Ovary	+	+	+	+	+	+	+	+	+	+	60
Granulosa cell tumor malignant											1
Uterus	+	+	+	+	+	+	+	+	+	+	60
Polyp stromal											1
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	59
Lymph node	+	+	+	+	+	+	+	+	+	+	60
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											2
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											3
Spleen	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear		X									3
Thymus	M	M	M	+	+	+	+	M	+	M	45
Integumentary System											
Mammary gland	+	M	+	M	+	+	+	+	+	+	54
Adenocarcinoma	X							X			5
Adenocarcinoma, multiple					X						1
Skin	+	+	+	+	+	+	+	+	+	+	59
Basal cell adenoma								X			3
Basal cell adenoma, multiple						X					2
Basal cell carcinoma											4
Keratoacanthoma							X				1
Papilloma squamous											5
Squamous cell carcinoma	X					X				X	7
Sebaceous gland, adenoma											1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	1 4 2	2 2 9	2 4 7	2 5 1	2 5 4	2 6 4	2 6 4	2 6 5	2 8 0	2 8 5	2 8 5	2 9 5	2 9 6	2 9 6	3 1 5	3 1 5	3 3 5	3 3 5	3 3 6	3 3 8	3 3 8	3 4 1	3 4 1	3 4 3
Carcass ID Number	0 9 3 5	0 9 3 4	1 0 2 5	1 0 2 4	0 9 2 5	0 9 5 5	1 0 5 5	0 9 4 5	0 9 4 4	1 0 1 4	1 0 1 5	0 9 9 5	0 9 6 5	0 9 8 5	0 9 7 4	0 9 7 5	1 0 2 3	1 0 3 5	0 9 6 4	0 9 4 3	0 9 6 3	0 9 8 4	0 9 4 2	0 9 6 2	0 9 5 3
Musculoskeletal System																								
Bone																								
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland												X						X				X		
Glioma malignant																								
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								
Alveolar/bronchiolar carcinoma																								
Carcinoma, metastatic, clitoral gland																								
Carcinoma, metastatic, uncertain primary site																								
Carcinoma, metastatic, Zymbal's gland													X											
Leukemia mononuclear																								
Squamous cell carcinoma, metastatic, pharynx																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Ear																								
Canal, papilloma squamous																								
Eye												+							+					
Conjunctiva, squamous cell																								
Harderian gland												+												
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X																				X
Carcinoma		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	
Bilateral, carcinoma																								
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								
Renal tubule, adenoma																								
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	3 4 3	3 4 3	3 5 5	3 5 5	3 6 4	3 7 0	3 7 0	3 7 7	3 7 8	3 8 3	3 8 3	3 8 4	3 8 4	3 9 0	3 9 1	3 9 1	3 9 1	3 9 1	3 9 1	4 0 5	4 0 5	4 0 5	4 0 6	4 0 6	4 0 6
Carcass ID Number	0 9 5 4	1 0 3 4	0 9 3 2	0 9 3 3	1 0 1 3	1 0 0 4	1 0 4 5	0 9 8 3	1 0 0 3	1 0 1 2	1 0 3 3	1 0 3 2	1 0 4 4	0 9 8 2	0 9 6 1	0 9 7 3	1 0 1 1	1 0 2 2	1 0 4 3	0 9 9 3	1 0 4 1	1 0 4 2	0 9 5 2	0 9 9 4	1 0 0 2
Musculoskeletal System																									
Bone									+																
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland																									
Glioma malignant										X															
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X															X		X		
Alveolar/bronchiolar carcinoma										X															
Carcinoma, metastatic, clitoral gland																			X					X	
Carcinoma, metastatic, uncertain primary site													X												
Carcinoma, metastatic, Zymbal's gland																									
Leukemia mononuclear					X																				
Squamous cell carcinoma, metastatic, pharynx																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Canal, papilloma squamous																									
Eye											+		+												
Conjunctiva, squamous cell													X												
Harderian gland																									
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma	X		X		X		X					X						X			X				
Carcinoma			X	X	X						X		X			X					X	X	X	X	X
Bilateral, carcinoma																				X					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X																				
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear					X																X	X			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride: 150 ppm (continued)

Number of Days on Study	4 2 1	4 2 1	4 2 1	4 2 1	4 2 1	4 2 2	4 2 2	4 2 2	4 2 2	4 2 6	
Carcass ID Number	0 9 4 1	0 9 5 1	0 9 7 1	0 9 7 2	0 9 8 1	0 9 9 1	0 9 9 2	1 0 2 1	1 0 3 1	1 0 0 1	Total Tissues/ Tumors
Musculoskeletal System											
Bone											1
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	60
Carcinoma, metastatic, Zymbal's gland											3
Glioma malignant											1
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+		60
Alveolar/bronchiolar adenoma											3
Alveolar/bronchiolar carcinoma											1
Carcinoma, metastatic, clitoral gland											2
Carcinoma, metastatic, uncertain primary site											1
Carcinoma, metastatic, Zymbal's gland											1
Leukemia mononuclear											1
Squamous cell carcinoma, metastatic, pharynx							X				1
Nose	+	+	+	+	+	+	+	+	+	+	60
Trachea	+	+	+	+	+	+	+	+	+	+	60
Special Senses System											
Ear										+	1
Canal, papilloma squamous										X	1
Eye											4
Conjunctiva, squamous cell											1
Harderian gland											1
Zymbal's gland	+	+	+	+	+	+	+	+	+	+	60
Adenoma	X		X						X		12
Carcinoma	X			X						X	34
Bilateral, carcinoma											1
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear											1
Renal tubule, adenoma							X				1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	60
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear		X									4

TABLE B3
Statistical Analysis of Primary Tumors in Female Rats in the 14-Month Drinking Water Study of 3,3'-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Clitoral Gland: Adenoma				
Overall rates[a]	0/60 (0%)	9/45 (20%)	32/75 (43%)	17/59 (29%)
Effective rates[b]	0/60 (0%)	9/45 (20%)	32/73 (44%)	17/58 (29%)
Terminal rates[c]	0/59 (0%)	5/39 (13%)	14/32 (44%)	5/10 (50%)
First incidence (days)		391	229	296
Life table tests[d]	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression tests[d]	P<0.001	P=0.003	P<0.001	P<0.001
Cochran-Armitage test[d]	P<0.001			
Fisher exact test[d]		P<0.001	P<0.001	P<0.001
Clitoral Gland: Carcinoma				
Overall rates	0/60 (0%)	5/45 (11%)	11/75 (15%)	18/59 (31%)
Effective rates	0/60 (0%)	5/45 (11%)	11/72 (15%)	18/55 (33%)
Terminal rates	0/59 (0%)	5/39 (13%)	3/32 (9%)	3/10 (30%)
First incidence (days)		421 (T)	315	254
Life table tests	P<0.001	P=0.010	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.010	P=0.004	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.013	P<0.001	P<0.001
Clitoral Gland: Adenoma or Carcinoma				
Overall rates	0/60 (0%)	14/45 (31%)	42/75 (56%)	32/59 (54%)
Effective rates	0/60 (0%)	14/45 (31%)	42/73 (58%)	32/58 (55%)
Terminal rates	0/59 (0%)	10/39 (26%	16/32 (50%)	7/10 (70%)
First incidence (days)		391	229	254
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
Large Intestine: Adenomatous Polyp				
Overall rates	0/60 (0%)	1/45 (2%)	6/75 (8%)	4/60 (7%)
Effective rates	0/60 (0%)	1/45 (2%)	6/70 (9%)	4/46 (9%)
Terminal rates	0/59 (0%)	1/39 (3%)	4/32 (13%)	1/10 (0%)
First incidence (days)		421 (T)	310	355
Life table tests	P<0.001	P=0.417	P=0.005	P<0.001
Logistic regression tests	P<0.019	P=0.417	P=0.036	P=0.012
Cochran-Armitage test	P=0.020			
Fisher exact test		P=0.429	P=0.022	P=0.033
Large Intestine: Adenomatous Polyp or Adenocarcinoma				
Overall rates	0/60 (0%)	1/45 (2%)	7/75 (8%)	4/60 (7%)
Effective rates	0/60 (0%)	1/45 (2%)	7/70 (10%)	4/46 (9%)
Terminal rates	0/59 (0%)	1/39 (3%)	4/32 (13%)	1/10 (10%)
First incidence (days)		421 (T)	310	355
Life table tests	P<0.001	P=0.417	P=0.002	P<0.001
Logistic regression tests	P=0.021	P=0.417	P=0.021	P=0.012
Cochran-Armitage test	P=0.021			
Fisher exact test		P=0.429	P=0.011	P=0.033

TABLE B3
Statistical Analysis of Primary Tumors in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Large Intestine (Colon): Adenomatous Polyp				
Overall rates	0/60 (0%)	1/45 (2%)	4/75 (5%)	2/60 (3%)
Effective rates	0/60 (0%)	1/45 (2%)	4/70 (6%)	2/46 (4%)
Terminal rates	0/59 (0%)	1/39 (3%)	3/32 (9%)	1/10 (10%)
First incidence (days)		421 (T)	310	355
Life table tests	P=0.009	P=0.417	P=0.023	P=0.046
Logistic regression tests	P=0.134	P=0.417	P=0.091	P=0.171
Cochran-Armitage test	P=0.136			
Fisher exact test		P=0.429	P=0.081	P=0.186
Small Intestine: Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	2/75 (3%)	5/60 (8%)
Effective rates	0/60 (0%)	0/45 (0%)	2/72 (3%)	5/57 (9%)
Terminal rates	0/59 (0%)	0/39 (0%)	0/32 (0%)	0/10 (0%)
First incidence (days)			309	251
Life table tests	P<0.001	–[e]	P=0.252	P<0.001
Logistic regression tests	P=0.009	–[e]	P=0.487	P=0.026
Cochran-Armitage test	P=0.003			
Fisher exact test		–[e]	P=0.296	P=0.025
Small Intestine: Adenomatous Polyp or Adenocarcinoma				
Overall rates	0/60 (0%)	1/45 (2%)	3/75 (5%)	5/60 (8%)
Effective rates	0/60 (0%)	1/45 (2%)	3/72 (4%)	5/57 (9%)
Terminal rates	0/59 (0%)	0/39 (0%)	1/32 (3%)	0/10 (0%)
First incidence (days)		391	309	251
Life table tests	P<0.001	P=0.437	P=0.095	P<0.001
Logistic regression tests	P=0.024	P=0.724	P=0.248	P=0.026
Cochran-Armitage test	P=0.011			
Fisher exact test		P=0.429	P=0.159	P=0.025
Small Intestine (Jejunum): Adenocarcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	2/75 (3%)	3/60 (5%)
Effective rates	0/60 (0%)	0/45 (0%)	2/71 (3%)	3/46 (7%)
Terminal rates	0/59 (0%)	0/39 (0%)	0/32 (0%)	0/10 (0%)
First incidence (days)			309	391
Life table tests	P=0.002	–[e]	P=0.252	P=0.006
Logistic regression tests	P=0.042	–[e]	P=0.487	P=0.029
Cochran-Armitage test	P=0.017			
Fisher exact test		–[e]	P=0.292	P=0.079
Liver: Neoplastic Nodule				
Overall rates	0/60 (0%)	0/45 (0%)	7/74 (9%)	3/60 (5%)
Effective rates	0/60 (0%)	0/45 (0%)	7/58 (12%)	3/36 (8%)
Terminal rates	0/59 (0%)	0/39 (0%)	6/32 (19%)	0/10 (0%)
First incidence (days)			378	343
Life table tests	P<0.001	–[e]	P<0.001	P=0.021
Logistic regression tests	P=0.015	–[e]	P=0.003	P=0.133
Cochran-Armitage test	P=0.014			
Fisher exact test		–[e]	P=0.006	P=0.050

TABLE B3
Statistical Analysis of Primary Tumors in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Liver: Neoplastic Nodule or Heptocellular Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	7/74 (9%)	4/60 (7%)
Effective rates	0/60 (7%)	0/45 (7%)	7/58 (12%)	4/36 (11%)
Terminal rates	0/59 (0%)	0/39 (0%)	6/32 (19%)	0/10 (0%)
First incidence (days)			378	343
Life table tests	P<0.001	–[e]	P<0.001	P=0.004
Logistic regression tests	P=0.003	–[e]	P=0.003	P=0.042
Cochran-Armitage test	P=0.004			
Fisher exact test		–[e]	P=0.006	P=0.018
Lung: Alveolar/Bronchiolar Adenoma				
Overall rates	1/60 (2%)	1/45 (2%)	3/74 (4%)	3/60 (5%)
Effective rates	1/60 (2%)	1/45 (2%)	3/63 (5%)	3/41 (7%)
Terminal rates	1/59 (2%)	1/39 (3%)	2/32 (6%)	0/10 (0%)
First incidence (days)	421 (T)	421 (T)	338	370
Life table tests	P=0.006	P=0.666	P=0.165	P=0.023
Logistic regression tests	P=0.086	P=0.666	P=0.369	P=0.130
Cochran-Armitage test	P=0.094			
Fisher exact test		P=0.676	P=0.328	P=0.181
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma				
Overall rates	1/60 (2%)	1/45 (2%)	3/74 (4%)	4/60 (7%)
Effective rates	1/60 (2%)	1/45 (2%)	3/63 (5%)	4/41 (10%)
Terminal rates	1/59 (2%)	1/39 (3%)	2/32 (6%)	0/10 (0%)
First incidence (days)	421 (T)	421 (T)	338	370
Life table tests	P=0.001	P=0.666	P=0.165	P=0.006
Logistic regression tests	P=0.032	P=0.666	P=0.369	P=0.060
Cochran-Armitage test	P=0.033			
Fisher exact test		P=0.676	P=0.328	P=0.086
Mammary Gland: Adenocarcinoma				
Overall rates	0/60 (0%)	1/45 (2%)	3/75 (4%)	6/60 (10%)
Effective rates	0/60 (0%)	1/45 (2%)	3/71 (4%)	6/51 (12%)
Terminal rates	0/59 (0%)	1/39 (3%)	1/32 (3%)	3/10 (30%)
First incidence (days)		421 (T)	363	285
Life table tests	P<0.001	P=0.417	P=0.084	P<0.001
Logistic regression tests	P=0.002	P=0.417	P=0.189	P=0.004
Cochran-Armitage test	P=0.002			
Fisher exact test		P=0.429	P=0.156	P=0.008
Mammary Gland: Fibroadenoma				
Overall rates	2/60 (3%)	1/45 (2%)	4/75 (5%)	0/60 (0%)
Effective rates	2/59 (3%)	1/39 (3%)	4/39 (10%)	0/13 (0%)
Terminal rates	2/59 (3%)	1/39 (3%)	3/32 (9%)	0/10 (0%)
First incidence (days)	421 (T)	421 (T)	406	
Life table tests	P=0.426	P=0.642N	P=0.122	P=0.665N
Logistic regression tests	P=0.549	P=0.642N	P=0.182	P=0.665N
Cochran-Armitage test	P=0.511			
Fisher exact test		P=0.652N	P=0.169	P=0.669N

TABLE B3
Statistical Analysis of Primary Tumors in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Mammary Gland: Fibroadenoma or Adenocarcinoma				
Overall rates	2/60 (3%)	2/45 (4%)	7/75 (9%)	6/60 (10%)
Effective rates	2/60 (3%)	2/45 (4%)	7/71 (10%)	6/51 (12%)
Terminal rates	2/59 (3%)	2/39 (5%)	4/32 (13%)	3/10 (30%)
First incidence (days)	421 (T)	421 (T)	363	285
Life table tests	P<0.001	P=0.538	P=0.017	P<0.001
Logistic regression tests	P=0.013	P=0.538	P=0.081	P=0.040
Cochran-Armitage test	P=0.046			
Fisher exact test		P=0.576	P=0.130	P=0.089
Oral Cavity (Tongue and Pharynx): Squamous Cell Papilloma				
Overall rates	0/60 (0%)	3/45 (7%)	7/75 (9%)	9/60 (15%)
Effective rates	0/60 (0%)	3/45 (7%)	7/73 (10%)	9/59 (15%)
Terminal rates	0/59 (0%)	3/39 (8%)	3/32 (9%)	3/10 (30%)
First incidence (days)		421 (T)	363	229
Life table tests	P=0.001	P=0.060	P=0.002	P<0.001
Logistic regression tests	P<0.001	P=0.060	P=0.013	P=0.002
Cochran-Armitage test	P=0.002			
Fisher exact test		P=0.076	P=0.013	P=0.001
Oral Cavity (Tongue and Pharynx): Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	1/45 (2%)	2/75 (3%)	4/60 (7%)
Effective rates	0/60 (0%)	1/45 (2%)	2/64 (3%)	4/41 (10%)
Terminal rates	0/59 (0%)	1/39 (3%)	2/32 (6%)	1/10 (10%)
First incidence (days)		421 (T)	421 (T)	338
Life table tests	P<0.001	P=0.417	P=0.118	P<0.007
Logistic regression tests	P=0.013	P=0.417	P=0.118	P=0.065
Cochran-Armitage test	P<0.008			
Fisher exact test		P=0.429	P=0.264	P=0.025
Oral Cavity (Tongue and Pharynx): Squamous Cell Papilloma or Carcinoma				
Overall rates	0/60 (0%)	3/45 (7%)	9/75 (12%)	13/60 (22%)
Effective rates	0/60 (0%)	3/45 (7%)	9/73 (12%)	13/59 (22%)
Terminal rates	0/59 (0%)	3/39 (8%)	5/32 (16%)	4/10 (40%)
First incidence (days)		421 (T)	363	229
Life table tests	P<0.001	P=0.060	P<0.001	P<0.001
Logistic regression tests	P=0.001	P=0.060	P=0.003	P<0.001
Cochran-armitage test	P<0.001			
Fisher exact test		P=0.076	P=0.004	P=0.001
Pharynx: Squamous Cell Papilloma				
Overall rates	0/60 (0%)	1/45 (2%)	5/75 (7%)	4/60 (7%)
Effective rates	0/60 (0%)	1/45 (2%)	5/73 (7%)	4/59 (7%)
Terminal rates	0/59 (0%)	1/39 (3%)	2/32 (6%)	2/10 (20%)
First incidence (days)		421 (T)	363	229
Life table tests	P<0.001	P=0.417	P=0.014	P=0.001
Logistic regression tests	P=0.033	P=0.417	P=0.048	P=0.062
Cochran-Armitage test	P=0.044			
Fisher exact test		P=0.429	P=0.047	P=0.057

TABLE B3
Statistical Analysis of Primary Tumors in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Pharynx: Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	2/75 (3%)	3/60 (5%)
Effective rates	0/60 (0%)	0/45 (0%)	2/59 (3%)	3/36 (8%)
Terminal rates	0/59 (0%)	0/39 (0%)	2/32 (6%)	1/10 (10%)
First incidence (days)			421 (T)	343
Life table tests	P<0.001	–[e]	P=0.118	P=0.015
Logistic regression tests	P=0.011	–[e]	P=0.118	P=0.094
Cochran-Armitage test	P=0.008			
Fisher exact test		–[e]	P=0.244	P=0.050
Pituitary Gland (Pars Distalis): Adenoma				
Overall rates	3/58 (5%)	1/45 (2%)	4/75 (5%)	0/60 (0%)
Effective rates	3/58 (5%)	1/44 (2%)	4/58 (7%)	0/31 (0%)
Terminal rates	3/57 (5%)	1/39 (3%)	2/32 (6%)	0/10 (0%)
First incidence (days)	421 (T)	421 (T)	363	
Life table tests	P=0.604N	P=0.449N	P=0.284	P=0.534N
Logistic regression tests	P=0.245N	P=0.449N	P=0.588	P=0.534N
Cochran-Armitage test	P=0.309N			
Fisher exact test		P=0.419N	P=0.500	P=0.272N
Skin: Basal Cell Adenoma				
Overall rates	0/60 (0%)	3/45 (7%)	5/75 (7%)	5/60 (8%)
Effective rates	0/60 (0%)	3/45 (7%)	5/64 (8%)	5/41 (12%)
Terminal rates	0/59 (0%)	3/39 (8%)	3/32 (9%)	2/10 (20%)
First incidence (days)		421 (T)	338	370
Life table tests	P<0.001	P=0.060	P=0.013	P<0.001
Logistic regression tests	P=0.009	P=0.060	P=0.062	P=0.002
Cochran-Armitage test	P=0.014			
Fisher exact test		P=0.076	P=0.034	P=0.009
Skin: Basal Cell Carcinoma				
Overall rates	0/60 (0%)	0/45 (0%)	5/75 (7%)	4/60 (7%)
Effective rates	0/60 (0%)	0/45 (0%)	5/69 (7%)	4/46 (9%)
Terminal rates	0/59 (0%)	0/39 (0%)	1/32 (3%)	0/10 (0%)
First incidence (days)			315	336
Life table tests	P<0.001	–[e]	P=0.015	P=0.010
Logistic regression tests	P=0.040	–[e]	P=0.063	P=0.098
Cochran-Armitage test	P=0.009			
Fisher exact test		–[e]	P=0.041	P=0.033
Skin: Basal Cell Adenoma or Basal Cell Carcinoma				
Overall rates	0/60 (0%)	3/45 (7%)	10/75 (13%	9/60 (15%)
Effective rates	0/60 (0%)	3/45 (7%)	10/69 (14%)	9/46 (20%)
Terminal rates	0/59 (0%)	3/39 (8%)	4/32 (13%)	2/10 (20%)
First incidence (days)		421 (T)	315	336
Life table tests	P<0.001	P=0.060	P<0.001	P<0.001
Logistic regression tests	P=0.001	P=0.060	P=0.005	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.076	P=0.001	P<0.001

TABLE B3
Statistical Analysis of Primary Tumors in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Skin: Squamous Cell Papilloma				
Overall rates	0/60 (0%)	1/45 (2%)	6/75 (8%)	5/60 (8%)
Effective rates	0/60 (0%)	1/45 (2%)	6/72 (8%)	5/55 (9%)
Terminal rates	0/59 (0%)	1/39 (3%)	4/32 (13%)	0/10 (0%)
First incidence (days)		421 (T)	391	264
Life table tests	P<0.001	P=0.417	P=0.003	P=0.002
Logistic regression tests	P=0.011	P=0.417	P=0.010	P=0.056
Cochran-Armitage test	P=0.015			
Fisher exact test		P=0.429	P=0.024	P=0.023
Skin: Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	2/45 (4%)	4/75 (5%)	7/60 (12%)
Effective rates	0/60 (0%)	2/45 (4%)	4/64 (6%)	7/41 (17%)
Terminal rates	0/59 (0%)	2/39 (5%)	3/32 (9%)	3/10 (30%)
First incidence (days)		421 (T)	406	338
Life table tests	P<0.001	P=0.153	P=0.016	P<0.001
Logistic regression tests	P<0.001	P=0.153	P=0.027	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P=0.068	P=0.001
Skin: Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rates	0/60 (0%)	3/45 (7%)	9/75 (12%)	12/60 (20%)
Effective rates	0/60 (0%)	3/45 (7%)	9/72 (13%)	12/55 (22%)
Terminal rates	0/59 (0%)	3/39 (8%)	6/32 (19%)	3/10 (30%)
First incidence (days)		421 (T)	391	264
Life table tests	P<0.001	P=0.060	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.060	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.076	P=0.003	P<0.001
Tongue: Squamous Cell Papilloma				
Overall rates	0/60 (0%)	2/45 (4%)	2/75 (3%)	7/60 (12%)
Effective rates	0/60 (0%)	2/45 (4%)	2/71 (3%)	7/51 (14%)
Terminal rates	0/59 (0%)	2/39 (5%)	1/32 (3%)	2/10 (20%)
First incidence (days)		421 (T)	391	285
Life table tests	P<0.001	P=0.153	P=0.149	P<0.001
Logistic regression tests	P<0.001	P=0.153	P=0.239	P=0.002
Cochran-Armitage test	P=0.001			
Fisher exact test		P=0.181	P=0.292	P=0.003
Tongue: Squamous Cell Papilloma or Carcinoma				
Overall rates	0/60 (0%)	2/45 (4%)	2/75 (3%)	8/60 (13%)
Effective rates	0/60 (0%)	2/45 (4%)	2/71 (3%)	8/51 (16%)
Terminal rates	0/59 (0%)	2/39 (5%)	1/32 (3%)	2/10 (20%)
First incidence (days)		421 (T)	391	285
Life table tests	P<0.001	P=0.153	P=0.149	P<0.001
Logistic regression tests	P<0.001	P=0.153	P=0.239	P=0.002
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P=0.292	P=0.001

TABLE B3
Statistical Analysis of Primary Tumors in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Uterus: Stromal Polyp				
Overall rates	11/60 (18%)	9/45 (20%)	3/75 (4%)	1/60 (2%)
Effective rates	11/60 (18%)	9/45 (20%)	3/64 (5%)	1/41 (2%)
Terminal rates	11/59 (19%)	8/39 (21%)	1/32 (3%)	0/10 (0%)
First incidence (days)	421 (T)	391	338	405
Life table tests	P=0.140N	P=0.397	P=0.168N	P=0.395N
Logistic regression tests	P=0.016N	P=0.471	P=0.032N	P=0.246N
Cochran-Armitage test	P=0.002N			
Fisher exact test		P=0.511	P=0.016N	P=0.013N
Uterus: Stromal Polyp or Sarcoma				
Overall rates	11/60 (18%)	10/45 (22%)	4/75 (5%)	1/60 (2%)
Effective rates	11/60 (18%)	10/45 (22%)	4/64 (6%)	1/41 (2%)
Terminal rates	11/59 (19%)	9/39 (23%)	2/32 (6%)	0/10 (0%)
First incidence (days)	421 (T)	391	338	405
Life table tests	P=0.183N	P=0.290	P=0.287N	P=0.395N
Logistic regression tests	P=0.024N	P=0.354	P=0.075N	P=0.246N
Cochran-Armitage test	P=0.002N			
Fisher exact test		P=0.400	P=0.036N	P=0.013N
Zymbal's Gland: Adenoma				
Overall rates	0/60 (0%)	4/45 (9%)	11/75 (15%)	12/60 (20%)
Effective rates	0/60 (0%)	4/45 (9%)	11/72 (15%)	12/57 (21%)
Terminal rates	0/59 (0%)	4/39 (10%)	5/32 (16%)	3/10 (30%)
First incidence (days)		421 (T)	338	251
Life table tests	P<0.001	P=0.024	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.024	P=0.003	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.031	P<0.001	P<0.001
Zymbal's Gland: Carcinoma				
Overall rates	0/60 (0%)	2/45 (4%)	22/75 (29%)	35/60 (58%)
Effective rates	0/60 (0%)	2/45 (4%)	22/74 (30%)	35/59 (59%)
Terminal rates	0/59 (0%)	0/39 (0%)	1/32 (3%)	3/10 (30%)
First incidence (days)		357	184	229
Life table tests	P<0.001	P=0.176	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.821	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.181	P<0.001	P<0.001
Zymbal's Gland: Adenoma or Carcinoma				
Overall rates	0/60 (0%)	6/45 (13%)	32/75 (43%)	42/60 (70%)
Effective rates	0/60 (0%)	6/45 (13%)	32/74 (43%)	42/59 (71%)
Terminal rates	0/59 (0%)	4/39 (10%)	6/32 (19%)	5/10 (50%)
First incidence (days)		357	184	229
Life table tests	P<0.001	P=0.005	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.023	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.005	P<0.001	P<0.001

TABLE B3
Statistical Analysis of Primary Tumors in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
All Organs: Leukemia (Lymphocytic, Monocytic, Mononuclear, or Undifferentiated)				
Overall rates	1/60 (2%)	3/45 (7%)	6/75 (8%)	4/60 (7%)
Effective rates	1/60 (2%)	3/44 (7%)	6/58 (10%)	4/31 (13%)
Terminal rates	0/59 (0%)	3/39 (8%)	4/32 (13%)	1/10 (10%)
First incidence (days)	390	421 (T)	363	364
Life table tests	P<0.001	P=0.181	P=0.015	P=0.007
Logistic regression tests	P=0.028	P=0.276	P=0.058	P=0.060
Cochran-Armitage test	P=0.031			
Fisher exact test		P=0.202	P=0.052	P=0.044
All Organs: Benign Tumors				
Overall rates	17/60 (28%)	24/45 (53%)	54/75 (72%)	41/60 (68%)
Effective rates	17/60 (28%)	24/45 (53%)	54/73 (74%)	41/59 (69%)
Terminal rates	17/59 (29%)	20/39 (51%)	28/32 (88%)	10/10 (100%)
First incidence (days)	421 (T)	391	229	229
Life table tests	P<0.001	P=0.003	P<0.001	P<0.001
Logistic regression tests	P<0.001	P=0.009	P<0.001	P<0.001
Cochran-Armitage test	P<0.001			
Fisher exact test		P=0.008	P<0.001	P<0.001
All Organs: Malignant Tumors				
Overall rates	1/60 (2%)	14/45 (31%)	49/75 (65%)	56/60 (93%)
Effective rates	1/60 (2%)	14/45 (31%)	49/74 (66%)	56/59 (95%)
Terminal rates	0/59 (0%)	11/39 (28%)	14/32 (44%)	9/10 (90%)
First incidence (days)	390	357	184	229
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
All Organs: Benign and Malignant Tumors				
Overall rates	18/60 (30%)	33/45 (73%)	71/75 (95%)	58/60 (97%)
Effective rates	18/60 (30%)	33/45 (73%)	71/74 (96%)	58/59 (98%)
Terminal rates	17/59 (29%)	27/39 (69%)	30/32 (94%)	10/10 (100%)
First incidence (days)	390	357	184	229
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

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined at site.
[b] Number of tumor-bearing animals/effective number of animals, i.e. number of animals alive at the 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 non-fatal. The Cochran-Armitage and Fisher exact tests compare directly the effective tumor rates. For all tests, a negative trend, or a lower incidence in a dose group is indicated by N.
[e] No tumors in dosed group or control group; statistical test not performed.

TABLE B4a
Historical Incidence of Tumors of the Large Intestine in Female F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls	
	Adenocarcinoma	Adenomatous Polyp or Adenocarcinoma
Historical Incidence at Hazleton Laboratories America, Inc.		
Decabromodiphenyl oxide	0/39	0/39
Chlorendic acid	0/49	0/49
Total	0/88 (0%)	0/88 (0%)
Overall Historical Incidence		
Total	0/1,601 (0.0%)	0/1,601 (0.0%)
Standard deviation	0.0%	0.0%
Range	0%–0%	0%-0%

[a] Data as of 22 November 1989, for studies of at least 104 weeks

TABLE B4b
Historical Incidence of Tumors of the Small Intestine in Female F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls	
	Adenocarcinoma	Adenomatous Polyp or Adenocarcinoma
Historical Incidence at Hazleton Laboratories America, Inc.		
Decabromodiphenyl oxide	0/49	0/49
Chlorendic acid	0/50	0/50
Total	0/99 (0%)	0/99 (0%)
Overall Historical Incidence		
Total	0/1,611 (0.0%)	0/1,611 (0.0%)
Standard deviation	0.0%	0.0%
Range	0%–0%	0%-0%

[a] Data as of 22 November 1989, for studies of at least 104 weeks

TABLE B4c
Historical Incidence of Liver Tumors in Female F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls		
	Neoplastic Nodule	Hepatocellular Carcinoma	Neoplastic Nodule or Hepatocellular Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.			
Decabromodiphenyl oxide	1/50	0/50	1/50
Chlorendic acid	1/50	0/50	1/50
Total	2/100 (2%)	0/100 (0%)	2/100 (2%)
Overall Historical Incidence			
Total	34/1,643 (2.1%)	3/1,643 (0.2%)	37/1,643 (2.3%)
Standard deviation	2.6 %	0.5%	2.7%
Range	0%–10%	0%–2%	0%–10%

[a] Data as of 22 November 1989, for studies of at least 104 weeks

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

Study	Incidence in Controls	
	Squamous Cell Papilloma	Squamous Cell Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.		
Decabromodiphenyl oxide	0/49	0/49
Chlorendic acid	0/50	0/50
Total	0/99 (0%)	0/99 (0%)
Overall Historical Incidence		
Total	1/1,643 (0.1%)	3/1,643 (0.2%)
Standard deviation	0.3%	0.5%
Range	0%–2%	0%–2%

[a] Data as of 22 November 1989, for studies of at least 104 weeks

TABLE B4e
Historical Incidence of Clitoral Gland Tumors in Female F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.			
Decabromodiphenyl oxide	0/50	4/50	4/50
Chlorendic acid	0/50	4/50	4/50
Total	0/100 (0%)	8/100 (8%)	8/100 (8%)
Overall Historical Incidence			
Total	62/1,643 (3.8%)	53/1,643 (3.2%)	115/1,643 (7.0%)
Standard deviation	4.3%	3.4%	4.8%
Range	0%–20%	0%–12%	0%–20%

[a] Data as of 22 November 1989, for studies of at least 104 weeks

TABLE B4f
Historical Incidence of Mammary Gland Tumors in Female F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls	
	Fibroadenoma	Adenocarcinoma
Historical Incidence at Hazleton Laboratories America, Inc.		
Decabromodiphenyl oxide	24/50	1/50
Chlorendic acid	22/50	1/50
Total	46/100 (2%)	2/100 (2%)
Overall Historical Incidence		
Total	510/1,643 (31.0%)	44/1,643 (2.7%)
Standard deviation	12.3%	2.2%
Range	10%–60%	0%-8%

[a] Data as of 22 November 1989, for studies of at least 104 weeks

TABLE B4g
Historical Incidence of Integumentary System Basal Cell Tumors in Female F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls		
	Basal Cell Tumor	Basal Cell Carcinoma	Basal Cell Tumor or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.			
Decabromodiphenyl oxide	0/50	0/50	0/50
Chlorendic acid	0/50	0/50	0/50
Total	0/100 (0%)	0/100 (0%)	0/100 (0%)
Overall Historical Incidence			
Total	2/1,643 (0.1%)	4/1,643 (0.2%)	6/1,643 (0.4%)
Standard deviation	0.4%	0.6%	0.7%
Range	0%–2%	0%–2%	0%–2%

[a] Data as of 22 November 1989, for studies of at least 104 weeks

TABLE B4h
Historical Incidence of Integumentary System Squamous Cell Tumors in Female F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.			
Decabromodiphenyl oxide	0/50	0/50	0/50
Chlorendic acid	0/50	0/50	0/50
Total	0/100 (0%)	0/100 (0%)	0/100 (0%)
Overall Historical Incidence			
Total	4/1,643 (0.2%)[b]	3/1,643 (0.2%)	7/1,643 (0.4%)
Standard deviation	0.4%	0.5%	0.8%
Range	0%–2%	0%–2%	0%–2%

[a] Data as of 22 November, 1989, for studies of at least 104 weeks
[b] Two (2) papillomas NOS are included in the incidence data.

TABLE B4i
Historical Incidence of Lung Tumors in Female F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls		
	Alveolar/Bronchiolar Adenoma	Alveolar/Bronchiolar Carcinoma	Alveolar/Bronchiolar Adenoma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.			
Decabromodiphenyl oxide	0/50	0/50	0/50
Chlorendic acid	0/50	0/50	0/50
Total	0/100 (0%)	0/100 (0%)	0/100 (0%)
Overall Historical Incidence			
Total	20/1,639 (1.2%)	5/1,639 (0.3%)	25/1,639 (1.5%)
Standard deviation	1.5%	0.7%	1.5%
Range	0%–6%	0%–2%	0%–6%

[a] Data as of 22 November, 1989, for studies of at least 104 weeks

TABLE B4j
Historical Incidence of Zymbal's Gland Tumors in Female F344/N Rats Receiving No Treatment[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Hazleton Laboratories America, Inc.			
Decabromodiphenyl oxide	0/50	0/50	0/50
Chlorendic acid	0/50	1/50	1/50
Total	0/100 (0%)	1/100 (1%)	1/100 (1%)
Overall Historical Incidence			
Total	1/1,643 (0.1%)	14/1,643 (0.9%)	14/1,643 (0.9%)
Standard deviation	0.0%	1.5%	1.5%
Range	0%–0%	0%–6%	0%–6%

[a] Data as of 22 November, 1989, for studies of at least 104 weeks

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

Study	Incidence in Controls	
	Glioma[b]	Astrocytoma
Historical Incidence at Hazleton Laboratories America, Inc.		
Decabromodiphenyl oxide	0/49	1/49
Chlorendic acid	0/50	0/50
Total	0/99 (0%)	1/99 (1%)
Overall Historical Incidence		
Total	0/1,628 (0.0%)	15/1,628 (0.9%)
Standard deviation	0.0%	1.5%
Range	0%–0%	0%–6%

[a] Data as of 22 November 1989, for studies of at least 104 weeks
[b] No reported incidence for this tumor morphology at the study laboratory.

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm	30 ppm	70 ppm	150 ppm
Disposition Summary				
Animals initially in study	70	45	75	70
Scheduled sacrifice	10			10
Early deaths				
Moribund	1	5	37	45
Dead		1	6	5
Survivors				
Terminal sacrifice	59	39	32	10
Animals examined microscopically	60	45	75	60
Alimentary System				
Intestine large, cecum	(60)	(45)	(75)	(60)
Edema				1 (2%)
Parasite metazoan			1 (1%)	
Intestine large, colon	(60)	(45)	(75)	(60)
Mineralization			2 (3%)	
Parasite metazoan	4 (7%)	2 (4%)	9 (12%)	6 (10%)
Intestine large, rectum	(60)	(45)	(74)	(60)
Parasite metazoan	6 (10%)	7 (16%)	5 (7%)	5 (8%)
Intestine small, jejunum	(60)	(45)	(74)	(59)
Muscularis, mineralization			1 (1%)	
Liver	(60)	(45)	(74)	(60)
Angiectasis			6 (8%)	2 (3%)
Basophilic focus		13 (29%)	11 (15%)	3 (5%)
Congestion				1 (2%)
Cyst				2 (3%)
Degeneration, chronic			1 (1%)	
Degeneration, cystic		3 (7%)	12 (16%)	11 (18%)
Eosinophilic focus		7 (16%)	57 (77%)	38 (63%)
Fatty change, multifocal			4 (5%)	2 (3%)
Hematocyst				1 (2%)
Hematopoietic cell proliferation		7 (16%)	19 (26%)	8 (13%)
Hemorrhage				1 (2%)
Hepatodiaphragmatic nodule	7 (12%)	7 (16%)	7 (9%)	3 (5%)
Hyperplasia, RE cell				1 (2%)
Inflammation, acute, multifocal			2 (3%)	
Inflammation, granulomatous, multifocal	11 (18%)	19 (42%)	10 (14%)	10 (17%)
Inflammation, suppurative, multifocal				1 (2%)
Mixed cell focus		34 (76%)	49 (66%)	32 (53%)
Necrosis, coagulative, focal		1 (2%)		1 (2%)
Necrosis, coagulative, multifocal			2 (3%)	
Necrosis, focal		2 (4%)	5 (7%)	1 (2%)
Bile duct, cyst			2 (3%)	1 (2%)
Bile duct, hyperplasia	20 (33%)	2 (4%)	1 (1%)	
Centrilobular, necrosis		1 (2%)		
Mesentery	(3)	(3)	(1)	
Hemorrhage		1 (33%)		
Artery, mineralization			1 (100%)	
Fat, necrosis, focal	3 (100%)	2 (67%)		

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Alimentary System (continued)				
Pancreas	(60)	(45)	(74)	(60)
Inflammation, chronic	1 (2%)			
Acinus, atrophy	5 (8%)	2 (4%)	1 (1%)	2 (3%)
Acinus, hyperplasia, focal			1 (1%)	
Artery, mineralization			1 (1%)	
Pharynx		(1)	(8)	(7)
Hyperplasia, focal			1 (13%)	
Salivary glands	(60)	(43)	(72)	(60)
Inflammation, chronic		1 (2%)	3 (4%)	1 (2%)
Stomach, forestomach	(60)	(45)	(74)	(60)
Hyperplasia, multifocal			1 (1%)	
Inflammation, chronic	1 (2%)			1 (2%)
Stomach, glandular	(60)	(45)	(74)	(60)
Degeneration, cystic	1 (2%)	3 (7%)	6 (8%)	11 (18%)
Erosion			2 (3%)	
Erosion, multifocal			1 (1%)	1 (2%)
Mineralization			5 (7%)	
Ulcer			1 (1%)	
Tongue		(2)	(3)	(10)
Hyperkeratosis, focal			1 (33%)	1 (10%)
Artery, mineralization			1 (33%)	
Tooth		(2)	(1)	
Periodontal tissue, inflammation, chronic active			2 (100%)	1 (100%)
Cardiovascular System				
Heart	(60)	(45)	(74)	(60)
Cardiomyopathy			3 (4%)	3 (5%)
Mineralization			4 (5%)	
Coronary artery, inflammation, chronic		1 (2%)		
Mitral valve, inflammation, suppurative				1 (2%)
Endocrine System				
Adrenal gland, cortex	(60)	(45)	(74)	(60)
Angiectasis			8 (11%)	8 (13%)
Cyst		1 (2%)		
Hematopoietic cell proliferation			1 (1%)	
Hyperplasia, focal		2 (4%)	3 (4%)	1 (2%)
Vacuolization cytoplasmic, focal	2 (3%)		10 (14%)	5 (8%)
Vacuolization cytoplasmic, multifocal				1 (2%)
Bilateral, angiectasis			1 (1%)	
Bilateral, hyperplasia, multifocal			1 (1%)	
Adrenal gland, medulla	(60)	(45)	(74)	(60)
Hyperplasia, focal			2 (3%)	
Parathyroid gland	(53)	(44)	(70)	(58)
Hyperplasia			5 (7%)	3 (5%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Endocrine System (continued)				
Pituitary gland	(58)	(45)	(75)	(60)
Angiectasis	1 (2%)	6 (13%)	3 (4%)	1 (2%)
Cyst	17 (29%)	7 (16%)	11 (15%)	8 (13%)
Hemorrhage		2 (4%)		
Pars distalis, hyperplasia				1 (2%)
Pars distalis, hyperplasia, focal	2 (3%)	1 (2%)	2 (3%)	1 (2%)
Thyroid gland	(60)	(45)	(74)	(60)
Ultimobranchial cyst	2 (3%)	1 (2%)	7 (9%)	5 (8%)
C-cell, hyperplasia		1 (2%)		
General Body System				
Tissue NOS			(6)	(3)
Mineralization			4 (67%)	
Genital System				
Clitoral gland	(60)	(45)	(75)	(59)
Hyperplasia		1 (2%)	3 (4%)	
Hyperplasia, squamous, focal			1 (1%)	
Inflammation, chronic active		1 (2%)		1 (2%)
Inflammation, suppurative			1 (1%)	1 (2%)
Bilateral, duct, ectasia		1 (2%)		
Duct, ectasia	6 (10%)	2 (4%)	12 (16%)	5 (8%)
Ovary	(60)	(44)	(75)	(60)
Cyst		2 (5%)		
Bilateral, parovarian tissue, cyst			1 (1%)	
Parovarian tissue, cyst	3 (5%)	4 (9%)	3 (4%)	2 (3%)
Uterus	(60)	(44)	(75)	(60)
Fibrosis			1 (1%)	
Hydrometra	5 (8%)	3 (7%)	1 (1%)	
Pigmentation, hemosiderin, focal		1 (2%)		
Thrombus				1 (2%)
Cervix, dilatation			1 (1%)	
Endometrium, cyst			3 (4%)	
Endometrium, hyperplasia, papillary				1 (2%)
Hematopoietic System				
Blood		(1)		
Anemia			1 (100%)	
Bone marrow	(60)	(45)	(73)	(59)
Atrophy			1 (1%)	4 (7%)
Hemorrhage			2 (3%)	4 (7%)
Hyperplasia				7 (12%)
Hyperplasia, RE cell, multifocal	1 (2%)			

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Hematopoietic System				
Lymph node (continued)	(60)	(45)	(75)	(60)
Hemorrhage				1 (2%)
Deep cervical, hyperplasia, plasma cell			1 (1%)	
Iliac, hyperplasia, plasma cell				1 (2%)
Inguinal, hyperplasia, plasma cell			1 (1%)	
Mediastinal, hemorrhage	21 (35%)	7 (16%)	4 (5%)	3 (5%)
Mediastinal, hyperplasia, plasma cell				2 (3%)
Mediastinal, pigmentation, hemosiderin		1 (2%)	2 (3%)	
Mediastinal, sinus, infiltration cellular, histiocytic			1 (1%)	
Renal, hemorrhage			2 (3%)	
Lymph node, mandibular	(60)	(43)	(72)	(60)
Hemorrhage	3 (5%)	2 (5%)	3 (4%)	2 (3%)
Hyperplasia, lymphoid	1 (2%)	2 (5%)	2 (3%)	2 (3%)
Hyperplasia, plasma cell	1 (2%)	5 (12%)	20 (28%)	18 (30%)
Sinus, ectasia	3 (5%)	3 (7%)	2 (3%)	1 (2%)
Lymph node, mesenteric	(60)	(45)	(73)	(60)
Atrophy			2 (3%)	1 (2%)
Hemorrhage			1 (1%)	
Spleen	(60)	(45)	(74)	(60)
Depletion lymphoid			1 (1%)	
Fibrosis			2 (3%)	
Fibrosis, focal			1 (1%)	
Hematopoietic cell proliferation		6 (13%)	20 (27%)	14 (23%)
Hemorrhage, multifocal	1 (2%)			
Pigmentation, hemosiderin			1 (1%)	
Red pulp, atrophy				2 (3%)
Thymus	(54)	(32)	(67)	(45)
Cyst		2 (6%)		1 (2%)
Depletion lymphoid				1 (2%)
Hemorrhage				1 (2%)
Epithelial cell, hyperplasia	7 (13%)	15 (47%)	17 (25%)	3 (7%)
Integumentary System				
Mammary gland	(56)	(42)	(73)	(54)
Fibrosis, focal	1 (2%)			
Duct, ectasia	2 (4%)	1 (2%)		
Skin	(60)	(45)	(75)	(59)
Acanthosis		3 (7%)		1 (2%)
Cyst epithelial inclusion			1 (1%)	3 (5%)
Inflammation, chronic active	2 (3%)			1 (2%)
Ulcer		1 (2%)		
Epidermis, hyperplasia, basal cell, focal			1 (1%)	
Hair follicle, hyperplasia, basal cell, focal			1 (1%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Month Drinking Water Study of 3,3'-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Musculoskeletal System				
Bone	(2)	(2)	(1)	
Cranium, fibrous osteodystrophy			2 (100%)	1 (100%)
Cranium, osteopetrosis		1 (50%)		
Sternum, fibrous osteodystrophy			1 (50%)	
Sternum, osteopetrosis		1 (50%)		
Nervous System				
Brain	(60)	(45)	(75)	(60)
Brain stem, compression	1 (2%)		1 (1%)	
Brain stem, hemorrhage			1 (1%)	
Cerebellum, hemorrhage				1 (2%)
Respiratory System				
Lung	(60)	(45)	(74)	(60)
Congestion			1 (1%)	2 (3%)
Edema				1 (2%)
Foreign body				1 (2%)
Hemorrhage			2 (3%)	2 (3%)
Infiltration cellular, histiocytic	12 (20%)	23 (51%)	36 (49%)	29 (48%)
Metaplasia, osseous, focal			1 (1%)	
Metaplasia, squamous			1 (1%)	
Mineralization			2 (3%)	
Pigmentation				1 (2%)
Alveolar epithelium, hyperplasia, focal	1 (2%)	7 (16%)	20 (27%)	7 (12%)
Alveolar epithelium, hyperplasia, multifocal		4 (9%)	10 (14%)	6 (10%)
Artery, mineralization			1 (1%)	
Bronchiole, inflammation, suppurative				1 (2%)
Interstitium, inflammation			4 (5%)	2 (3%)
Peribronchiolar, alveolus, inflammation, suppurative			1	(2%)
Nose	(60)	(44)	(75)	(60)
Foreign body				1 (2%)
Fungus			1 (1%)	
Hemorrhage	5 (8%)		2 (3%)	4 (7%)
Inflammation, suppurative			6 (8%)	5 (8%)
Olfactory epithelium, hyperplasia, focal			1 (1%)	
Special Senses System				
Ear			(1)	
Pinna, ulcer				1 (100%)
Eye	(1)	(4)	(4)	
Anterior chamber, hemorrhage			2 (50%)	
Cornea, inflammation, suppurative			3 (75%)	3 (75%)
Harderian gland			(1)	(1)
Pigmentation, porphyrin			1 (100%)	1 (100%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride (continued)

	0 ppm	30 ppm	70 ppm	150 ppm
Special Senses System (continued)				
Zymbal's gland	(57)	(44)	(73)	(60)
Cyst		1 (2%)		1 (2%)
Ectasia			4 (5%)	
Hyperplasia, glandular, focal		3 (7%)	3 (4%)	
Hyperplasia, squamous, focal		1 (2%)	4 (5%)	2 (3%)
Hypertrophy			3 (4%)	3 (5%)
Inflammation, chronic active			1 (1%)	
Urinary System				
Kidney	(60)	(45)	(74)	(60)
Hemorrhage				1 (2%)
Karyomegaly		28 (62%)	61 (82%)	56 (93%)
Nephropathy	47 (78%)	44 (98%)	72 (97%)	59 (98%)
Medulla, inflammation, suppurative				1 (2%)
Urinary bladder	(60)	(45)	(75)	(60)
Dilatation			2 (3%)	
Hemorrhage			1 (1%)	
Hyperplasia, papillary				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 Zeiger *et al.* (1988). The test chemical was sent to the laboratories as coded aliquots from the Radian Corporation, Austin, TX. The test chemical was incubated with the *Salmonella typhimurium* tester strain (TA97, TA98, TA100, or TA1535) 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 by toxicity to 666 μg/plate. Tests were repeated for all negative assays and all positive assays were retested under the conditions which 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 which 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.

CHINESE HAMSTER OVARY CYTOGENETICS ASSAYS

Testing was performed as reported by Galloway *et al.* (1987) and is briefly described as follows. The test chemical was sent to the laboratories as coded aliquots from Radian Corporation, Austin, TX. The test chemical was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) 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 test chemical; the high dose was limited by toxicity and did not exceed 500 μg.

In the SCE test without S9, CHO cells were incubated for 26 hours with the test 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 test 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 test 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

test 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.

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 SCEs 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).

DROSOPHILA MELANOGASTER PROTOCOL

Two studies using *Drosophila melanogaster* were performed at the University of Wisconsin (Madison, WI). The first study investigated sex-linked recessive lethal mutations in the *D. melanogaster* induced by the test chemical; the second investigated induction of reciprocal translocations. These assays for gene mutation and chromosomal translocation induction were performed as described by Valencia *et al.* (1985). Test chemical was supplied as coded aliquots from the Radian Corporation, Austin, TX. The test chemical was assayed in the sex-linked recessive lethal (SLRL) test by feeding to Canton-S males that were no more than 24 hours old. The test chemical was also tested by injection into the adult male *D. melanogaster*. Because administration of the test chemical produced positive results for SLRL tests, the test chemical was also assayed for induction of reciprocal translocations (RTs) using the same method of exposure.

The test chemical was administered with a glass Pasteur pipette drawn out in a flame to a filament with the tip broken to provide an opening for delivery of the test solution. Injections were performed either manually by attaching a rubber bulb to the opposite end of the pipette and forcing through sufficient solution (0.2-0.3 μL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector which automatically delivered a calibrated volume. Flies were anesthetized with ether and immobilized on a strip of double-stick tape. The chemical was injected under the wing into the thorax with the aid of a dissecting microscope.

Toxicity tests attempted to set concentrations of test chemical at a level that would produce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. For the SLRL tests, Canton-S males (10-20 flies/vial) were allowed to feed for 72 hours on a solution of the test chemical (14,000 ppm) in 5% sucrose. In the injection experiments, 24- to 72-hour-old Canton-S males were given a solution of the test chemical (2,750 ppm) formulated with 0.7% saline and allowed to recover for 24 hours. Exposed males were mated with three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings at 3, 2, and 2 days; sample sperm from successive matings were treated as spermatozoa (mating 1), spermatids (mating 2), and spermatocytes (mating 3). F_1 heterozygous females were mated with their siblings and

then were placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution). When a cluster was identified, all data from the male in question was discarded. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. The two experiments, using feeding and injection, resulted in the testing of approximately 13,000 treated and 15,000 control chromosomes. Results were significant for SLRL at the 5% level (Margolin *et al.*, 1983).

Based on positive results for the SLRL tests, the test chemical was also assayed for induction of reciprocal translocations. A detailed protocol of the sex-linked recessive assay is presented in Valencia *et al.* (1985). Exposure of Canton-S males to the test chemical was by feed, similar to that described for the SLRL tests. Exposed males were mated to three bw; e females for three days and then discarded. The females were transferred to fresh medium at 3- to 4-day intervals to produce a total of six cultures; the females were then discarded. This allowed successive cultures to sample sperm that were stored for increasing lengths of time. Individual F_1 females were backcrossed to bw; e females, and the F_2 were screened for pseudolinkage. This procedure allowed the recovery of translocations involving the Y, second, or third chromosomes in any combination. Presumptive translocations were retested. A total of 4,794 F_1 female flies were tested and no translocations were recognized. Thus, results were not significant for RTs at the 5% level (Kastenbaum and Bowman, 1970).

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

RESULTS

3,3′-Dimethylbenzdine produced positive responses at low doses in several tests for genotoxicity. 3,3′-Dimethylbenzidine dihydrochloride was tested for induction of gene mutations in *Salmonella typhimurium* strains TA100, TA1535, TA97, and TA98 using a preincubation protocol in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Zeiger *et al.*, 1988). Mutagenic activity was observed only in strain TA98 in the presence of S9 (Table C1). In cytogenetic tests with Chinese hamster ovary (CHO) cells, 3,3′-dimethylbenzidine induced both sister-chromatid exchanges (SCE) (Table C2) and chromosomal aberrations (Table C3) in the absence of S9. Neither endpoint was elevated in trials conducted with S9 from Aroclor 1254-induced male Sprague-Dawley rat liver. In the SCE tests, positive responses were recorded in each of two trials without S9. In the chromosomal aberration assay, the first of two trials without S9 was negative, but in the second trial, three intermediate dose levels produced significant increases in aberrations. 3,3′-Dimethylbenzidine induced sex-linked recessive lethal mutations in the germ cells of male *Drosophila melanogaster* when administered either by feeding or by injection (Table C4). No induction of reciprocal translocations occurred in *D. melanogaster* germ cells following exposure of males by feeding (Table C5).

Table C1
Mutagenicity of 3,3′-Dimethylbenzidine Dihydrochloride in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9	+S9 (hamster)	+S9 (rat)
TA100	0	103 ± 4.6	86 ± 0.5	102 ± 6.2
	10	87 ± 6.4	87 ± 5.2	116 ± 7.7
	33	90 ± 3.5	106 ± 3.8	118 ± 1.7
	100	95 ± 7.5	118 ± 10.9	116 ± 4.8
	333	91 ± 2.0	118 ± 0.9	105 ± 3.6
	666	86 ± 2.6[c]	114 ± 7.4[c]	118 ± 8.5[c]
Trial summary		Negative	Equivocal	Negative
Positive control[d]		474 ± 26.2	305 ± 6.5	733 ± 9.3
TA1535	0	17 ± 3.7	8 ± 0.7	9 ± 1.5
	10	16 ± 2.0	6 ± 1.2	6 ± 1.5
	33	15 ± 1.7	6 ± 2.7	13 ± 1.8
	100	12 ± 1.5	9 ± 1.5	9 ± 1.5
	333	14 ± 1.5	8 ± 0.7	7 ± 1.8
	666	6 ± 0.3[c]	8 ± 2.4[c]	8 ± 1.0[c]
Trial summary		Negative	Negative	Negative
Positive control[d]		209 ± 6.4	43 ± 1.3	169 ± 11.3
TA97	0	86 ± 6.2	116 ± 3.8	133 ± 2.0
	10	91 ± 3.3	114 ± 1.2	176 ± 1.0
	33	91 ± 3.0	119 ± 6.8	130 ± 2.6
	100	88 ± 9.5	124 ± 10.6	142 ± 8.5
	333	71 ± 5.9	122 ± 8.4	121 ± 9.0
	666	52 ± 4.6[c]	101 ± 3.5[c]	110 ± 9.4[c]
Trial summary		Negative	Negative	Negative
Positive control[d]		461 ± 33.3	228 ± 11.5	1178 ± 49.5
TA98	0	18 ± 2.9	29 ± 0.3	30 ± 7.1
	10	15 ± 2.5	59 ± 0.9	64 ± 1.9
	33	15 ± 2.3	74 ± 2.6	92 ± 3.7
	100	17 ± 1.2	88 ± 7.3	101 ± 4.4
	333	13 ± 2.3	133 ± 1.7	100 ± 8.8
	666	10 ± 3.4[c]	163 ± 15.9[c]	97 ± 2.7[c]
Trial summary		Negative	Positive	Positive
Positive control[d]		145 ± 3.4	225 ± 7.8	286 ± 28.9

[a] Study performed at Microbiological Associates, Inc. The detailed protocol is presented in Zeiger et al. (1988). Cells and study compound or solvent (distilled water) were incubated in the absence of exogenous metabolic activations (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity; 0 μg/plate dose is the solvent control.

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

[c] Slight toxicity

[d] 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 TA97.

Table C2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 3,3′-Dimethylbenzidine Dihydrochloride[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: Weak positive								
Dimethylsulfoxide		50	1,033	413	0.39	8.3	26.0	
Mitomycin-C	0.0050	50	1,049	1578	1.50	31.6	26.0	276.26
3,3'-Dimethylbenzidine Dihydrochloride								
	0.5	50	1,044	420	0.40	8.4	26.0	0.62
	1.6	50	1,047	429	0.40	8.6	26.0	2.49
	5.0	50	1,038	451	0.43	9.0	26.0	8.67
	16.0	50	1,047	486	0.46	9.7	26.0	16.10
	50.0	50	1,039	638	0.61	12.8	26.0	53.59*
								P<0.001[d]
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,041	479	0.46	9.6	26.0	
Mitomycin-C	0.005	50	1,050	1393	1.32	27.9	26.0	188.32
	0.010	50	1,045	2049	1.96	41.0	26.0	326.13
3,3'-Dimethylbenzidine Dihydrochloride								
	5.0	50	1,046	465	0.44	9.3	26.0	-3.39
	10.0	50	1,044	576	0.55	11.5	26.0	19.91
	20.0	50	1,038	605	0.58	12.1	26.0	26.67*
	40.0	50	1,035	767	0.74	15.3	26.0	61.05*
	60.0	50	1,041	862	0.82	17.2	26.0	79.96*
								P<0.001
+S9[e]								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,042	464	0.44	9.3	26.0	
Cyclophosphamide	2.000	50	1,047	3560	3.40	71.2	26.0	663.59
3,3'-Dimethylbenzidine Dihydrochloride								
	1.6	50	1,044	417	0.39	8.3	26.0	-10.30
	5.0	50	1,049	435	0.41	8.7	26.0	-6.88
	16.0	50	1,039	460	0.44	9.2	26.0	-0.58
	50.0	50	1,032	407	0.39	8.1	26.0	-11.44
	160.0	50	1,038	439	0.42	8.8	26.0	-5.02
	500.0	50	1,037	484	0.46	9.7	26.0	4.81
								P=0.197

TABLE C2
Induction of Sister-Chromatid Exchanges in Chinese Hamster Ovary Cells by 3,3′-Dimethylbenzidine Dihydrochloride (continued)

* Positive (≥ 20% increase over solvent control)

[a] Study performed at Environmental Health Research and Testing, Inc. SCE = sister chromatid excahnge; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1987). Briefly, Chinese hamster overy cells were incubated with test study compound or solvent (dimethylsulfoxide) 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 compound relative to those of culture exposed to solvent. Values at least 20% above control levels are considered significant and are indicated by an asterisk (*).

[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 2-3 hours.

[d] Significance of relative SCEs/chromosomes tested by linear regression 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 2-3 hours. S9 was from the livers of Aroclor 1,254-induced male Sprague Dawley rats.

TABLE C3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 3,3′-Dimethylbenzidine Dihydrochloride[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: 12.0 h					Harvest time: 14.0 h				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	0	0.00	0.0		100	0	0.00	0.0
Mitomycin-C					Cyclophosphamide				
0.25	100	30	0.30	24.0	50	100	104	1.04	60.0
3,3'-Dimethylbenzidine Dihydrochloride					3,3'-Dimethylbenzidine Dihydrochloride				
5	100	2	0.02	2.0	5	100	0	0.00	0.0
16	100	4	0.04	4.0	16	100	1	0.01	1.0
50	100	0	0.00	0.0	50	100	0	0.00	0.0
160	100	1	0.01	1.0	160	100	1	0.01	1.0
Summary: Negative					Summary: Negative				
				P=0.497					P=0.157
Trial 2									
Harvest time: 12.0 h									
Dimethylsulfoxide									
	100	0	0.00	0.0					
Mitomycin-C									
0.5	100	37	0.37	31.0					
3,3'-Dimethylbenzidine Dihydrochloride									
5	100	5	0.05	5.0					
10	100	7	0.07	6.0*					
20	100	14	0.14	12.0*					
40	100	22	0.22	17.0*					
60	100	0	0.00	0.0					
80	100	1	0.01	1.0					
100	100	1	0.01	1.0					
Summary: Positive									
				P=0.532					

TABLE C3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 3,3′-Dimethylbenzidine Dihydrochloride (continued)

* Statistically significant dose point ($P<0.05$).
[a] Study performed at Environmental Health Research and Testing, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (dimethylsulfoxide) as indicated in [b] and [c]. Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake off, fixed, and stained in 6% Giemsa.
[b] In the absence of S9, cells were incubated with study compound or solvent for 8-10 hours at 37° C. Cells were then washed and fresh medium containing Colcemid was added for an additional 2-3 hours followed by harvest.
[c] In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the livers of Aroclor 1,254-induced male Sprague Dawley rats.
[d] Significance of percent cells with aberrations tested by linear regression trend test vs. log of the dose

TABLE C4
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by 3,3′-Dimethylbenzidine Dihydrochloride[a]

Route of Exposure	Dose (ppm)	Incidence of Deaths (percent)	Incidence of Sterility (percent)	No. of Lethals/No. of X Chromosomes Tested Mating 1	Mating 2	Mating 3	Overall Total[b]
Feeding	14,000	17	0	5/2,279	1/1,591	3/1,850	9/5,720 (0.16%)
	0			0/3,449	1/3,139	1/3,069	2/9,657 (0.02%)
Injection	2,750	24	0	4/2,653	1/2,439	4/2,007	9/7,099 (0.13%)
	0			0/2,334	1/2,179	1/1,669	2/6,182 (0.03%)

[a] Study performed at the University of Wisconsin-Madison. A detailed protocol of the sex-linked recessive lethal assay is presented in Valencia *et al.* (1985). Exposure by feeding was done by allowing 24-hour-old Canton-S males to feed for 3 days on a solution of the study compound dissolved in 5% sucrose. In the injection experiments, 24-hour-old Canton-S males were treated with a solution of the study compound dissolved in 0.7% saline and allowed 24 hours to recover. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three broods of 3, 2, and 2 days; sample sperm from successive matings were treated as spermatozoa (mating 1), spermatids (mating 2), and spermatocytes (mating 3). F_1 heterozygous females were crossed to their siblings and placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters; clusters were removed in the injection experiment. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. Results were significant at the 5% level (Margolin *et al.*, 1983).

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

TABLE C5
Induction of Reciprocal Translocations in *Drosophila melanogaster* by 3,3′-Dimethylbenzidine Dihydrochloride[a]

Route of Exposure	Dose (ppm)	Transfers Translocations/Total F_1 Tested 1	2	3	4	5	6	No. of Tests	Total No. of Translocations	Total Translocations (%)
Feeding	14,000	0/857	0/820	0/836	0/833	0/819	0/629	4,794	0	0.00
Concurrent control								32,516	1	0.00
Historical control								116,163	2	0.00

[a] Study performed at the University of Wisconsin-Madison. A detailed protocol of the reciprocal translocation assay is presented in Valencia *et al.* (1985). Exposed males were mated to three bw;e females for three days and discarded. The females were transferred to fresh medium every 3-4 days to produce a total of six cultures, and then they were discarded. In this manner, sample sperm from successive cultures were stored for increasing lengths of time. Individual F_1 males were backcrossed to bw;e females, and the F_2 were screened for pseudolinkage. This procedure allows the recovery of translocations involving the Y, second, or third chromosomes in any combination. Presumptive translocations were retested. Results were not significant at the 5% level (Kastenbaum and Bowman, 1970).

APPENDIX D
HEMATOLOGY, CLINICAL CHEMISTRY, AND URINALYSIS RESULTS IN THE 13-WEEK AND 9-MONTH DRINKING WATER STUDIES

TABLE D1
Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride[a]

Analysis	0 ppm	300 ppm	500 ppm	1,000 ppm	2,000 ppm[b]
Male					
Hematocrit (%)	45.2 ± 0.54	48.2 ± 0.76	44.8 ± 0.74	40.3 ± 0.79**	40.4 ± 0.57**
Hemoglobin (g/dL)	16.1 ± 0.15	16.3 ± 0.17	16.0 ± 0.24	15.9 ± 0.15	16.0 ± 0.22
Erythrocytes (10^6/μL)	8.78 ± 0.094	9.15 ± 0.132	8.59 ± 1.151	7.90 ± 0.146**	7.90 ± 0.105**
Leukocytes (10^3/μL)	5.4 ± 0.146	5.0 ± 0.264	5.4 ± 0.197	6.7 ± 0.363**	7.0 ± 0.732*
Segmented neutrophils (10^3/μL)	1.04 ± 0.097	1.07 ± 0.091	1.10 ± 0.094	1.42 ± 0.158	1.14 ± 0.269
Lymphocytes (10^3/μL)	3.96 ± 0.256	3.82 ± 0.219	4.12 ± 0.200	5.02 ± 0.240*	5.57 ± 0.510*
Monocytes (10^3/μL)	0.13 ± 0.023	0.08 ± 0.022	0.12 ± 0.018	0.15 ± 0.030	0.30 ± 0.064*
Eosinophils (10^3/μL)	0.04 ± 0.018	0.05 ± 0.008	0.04 ± 0.016	0.06 ± 0.020	0.03 ± 0.013
BUN (mg/dL)	17.9 ± 0.62	18.5 ± 0.81	18.8 ± 0.80	20.4 ± 0.99	25.0 ± 3.08*
Creatinine (mg/dL)	0.62 ± 0.020	0.57 ± 0.026	0.62 ± 0.013	0.62 ± 0.013	0.57 ± 0.021
LDH (IU/L)	590 ± 49.47	762 ± 59.90*	663 ± 52.99	1018 ± 36.62**	623 ± 53.88
SDH (IU/L)	8.7 ± 0.616	13.8 ± 0.854**	26.5 ± 4.145**	32.7 ± 2.511**	14.3 ± 1.498**
ALT (mg/dL)	40 ± 2.46	33 ± 1.42	47 ± 5.99	54 ± 3.89*	43 ± 5.85
T_3 (ng/dL)[c]	62.12 ± 5.35	59.65 ± 3.94	67.84 ± 3.55	69.73 ± 4.03	58.80 ± 5.69
T_4 (μg/dL)[c]	4.73 ± 0.178	3.06 ± 0.158**	2.98 ± 0.181**	3.06 ± 0.136**	2.80 ± 0.163**
TSH (ng/mL)[c]	551.50 ± 37.99	496.60 ± 54.49	511.30 ± 27.82	502.10 ± 37.75	492.25 ± 56.22
Female					
Hematocrit (%)	48.3 ± 0.68	46.1 ± 0.69	44.6 ± 0.72**	41.4 ± 0.95**	37.6 ± 0.78**
Hemoglobin (g/dL)	16.3 ± 0.16	16.0 ± 0.13	15.8 ± 0.19	15.8 ± 0.21	15.4 ± 0.42*
Erythrocytes (10^6/μL)	8.86 ± 0.098	8.06 ± 0.262**	8.13 ± 0.131**	7.58 ± 0.175**	7.07 ± 0.173**
Leukocytes (10^3/μL)	4.3 ± 0.152	4.2 ± 0.292	4.9 ± 0.376	5.7 ± 0.283**	5.9 ± 0.658*
Segmented neutrophils (10^3/μL)	1.00 ± 0.083	0.54 ± 0.042**	0.75 ± 0.089	1.11 ± 0.132	0.57 ± 0.071*
Lymphocytes (10^3/μL)	3.25 ± 0.116	3.64 ± 0.284	4.11 ± 0.334	4.50 ± 0.186**	4.86 ± 0.409**
Monocytes (10^3/μL)	0.02 ± 0.012	0.00 ± 0.004	0.02 ± 0.015	0.02 ± 0.009	0.02 ± 0.011
Eosinophils (10^3/μL)	0.04 ± 0.012	0.01 ± 0.006	0.05 ± 0.022	0.03 ± 0.013	0.01 ± 0.010
BUN (mg/dL)	20.5 ± 0.65	18.9 ± 0.62	20.8 ± 0.79	21.8 ± 1.35	30.0 ± 5.32
Creatinine (mg/dL)	0.60 ± 0.021	0.48 ± 0.013**	0.51 ± 0.018	0.53 ± 0.015	0.70 ± 0.073
LDH (IU/L)	456 ± 19.32	640 ± 40.51*	628 ± 47.61*	494 ± 46.00	517 ± 66.93
SDH (IU/L)	5.5 ± 0.654	9.4 ± 0.581**	16.0 ± 2.708**	14.8 ± 1.504**	13.0 ± 1.612**
ALT (mg/dL)	30 ± 1.4	27 *± 1.28	30 ± 2.08	37 ± 2.74	51 ± 7.86**
T_3 (ng/dL)[c]	102.42 ± 3.11	72.58 ± 4.48**	69.58 ± 5.26**	55.24 ± 3.50**	47.43 ± 5.60**
T_4 (μg/dL)[c]	2.50 ± 0.091	1.87 ± 0.092**	1.77 ± 0.131**	1.69 ± 0.087**	1.95 ± 0.198**
TSH (ng/mL)[c]	496.40 ± 34.11	728.70 ± 231.27	574.70 ± 74.88	620.30 ± 72.94	464.00 ± 20.19

* 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.
[b] Six animals were examined.
[c] T_3 and T_4 were analyzed with the Tri-Tab Diagnostic Kit and the Tetra-Tab RIA Diagnostic Kit (Nuclear Medical Laboratories). TSH analysis was performed by the method of Ridgway *et al.* (1973).

TABLE D2
Hematology, Clinical Chemistry, and Urinalysis Data for F344/N Rats in the 9-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride[a]

Analysis	Male		Female	
	0 ppm	150 ppm	0 ppm	150 ppm
Hematocrit (%)	43.9 ± 0.71	35.5 ± 1.06**	45.6 ± 0.72	34.5 ± 0.51**
Hemoglobin (g/dL)	16.6 ± 0.16	15.1 ± 0.34**	16.1 ± 0.12	14.7 ± 0.33**
Erythrocytes (10^6/μL)	8.61 ± 0.13	6.90 ± 0.22**	8.26 ± 0.11	6.10 ± 0.08**
Nucleated erythrocytes/leukocytes x 10^2	0.100 ± 0.100	0.200 ± 0.200	0.200 ± 0.133	0.100 ± 0.100
Leukocytes (10^3/μL)	8.0 ± 0.21	10.0 ± 0.64**	4.4 ± 0.39	7.2 ± 0.72**
Segmented neutrophils (%)	43.40 ± 1.75	52.00 ± 4.58	36.30 ± 2.53	40.10 ± 4.31
Lymphocytes (%)	53.30 ± 1.65	46.50 ± 4.46	60.80 ± 2.62	58.50 ± 4.09
Monocytes (%)	1.70 ± 0.33	1.30 ± 0.21	1.00 ± 0.26	0.90 ± 0.41
Eosinophils (%)	1.50 ± 0.27	0.20 ± 0.13**	1.90 ± 0.43	0.50 ± 0.22*
MCH (pg)[b]	19.3 ± 0.20	22.0 ± 0.40**	19.5 ± 0.27	24.2 ± 0.39**
MCHC (%)	37.6 ± 0.42	42.7 ± 0.73**	35.4 ± 0.55	42.8 ± 0.79**
MCV (μ^3)	51.0 ± 0.18	51.5 ± 0.32	55.1 ± 0.23	56.4 ± 0.19**
BUN (mg/dL)	18.7 ± 1.17	28.6 ± 5.33*	16.9 ± 0.43	14.9 ± 1.21**
Creatinine (mg/dL)	0.69 ± 0.05	1.00 ± 0.13*	0.64 ± 0.02	0.65 ± 0.02
Serum glucose (mg/dL)	147 ± 5.6	150 ± 5.8	120 ± 3.2	190 ± 18.1**
ALT (IU/L)	73.3 ± 9.87	85.1 ± 4.78*	30.2 ± 3.70	98.6 ± 42.5**
LDH (IU/L)	1053 ± 103	894 ± 165	297 ± 13.5	343 ± 62.5
SDH (IU/L)	14.8 ± 1.90	31.1 ± 3.86**	7.1 ± 1.43	71.4 ± 40.24**
T_3 (ng/dL)[c]	81.7 ± 8.45	114.0 ± 8.16*	104.8 ± 3.88	94.7 ± 4.67
T_4 (μg/dL)[c]	3.5 ± 0.26	2.2 ± 0.30**	3.44 ± 0.15	2.44 ± 0.20**
TSH (ng/mL)[c]	337.6 ± 25.3	501.3 ± 54.5*	321.1 ± 23.4	486.2 ± 73.6*
Serum osmolality (MOS/kg)	321 ± 4.3	323 ± 2.7	316 ± 2.7	320 ± 3.0
Urine osmolality (MOSM/kg)	1350 ± 271	2730 ± 303*	1568 ± 246	2247 ± 164*
Osmolality ratio (urine/serum)	4.21 ± 0.86	8.44 ± 0.92*	4.96 ± 0.77	7.03 ± 0.53*
Urine creatinine (mg/dL)	163.7 ± 32.1	271.2 ± 26.0*	151.3 ± 21.3	245.4 ± 14.1**
Urine volume (mL/16 h)	7.3 ± 1.24	2.5 ± 0.53**	3.1 ± 0.46	0.9 ± 0.14**
Urine specific gravity	1.03 ± 0.00	1.06 ± 0.00**	1.04 ± 0.00	1.06 ± 0.00**
Urine pH	6.65 ± 0.11	6.50 ± 0.00	6.55 ± 0.09	6.55 ± 0.09
Urine protein (mg/dL)	51.00 ± 10.69	300.00 ± 0.00**	45.00 ± 12.58	300.00 ± 0.00**
Creatinine excretion rate (mg/16 h)	8.91 ± 0.33	5.57 ± 0.49**	3.71 ± 0.32	2.07 ± 0.32**

* Significantly different ($P \leq 0.05$) from the control group by Wilcoxon's test (Hollander and Wolfe, 1973)
** $P \leq 0.01$
[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.
[b] Rank transformed data analyzed.
[c] T_3 and T_4 were analyzed with the Tri-Tab Diagnostic Kit and the Tetra-Tab RIA Diagnostic Kit (Nuclear Medical Laboratories). TSH analysis was performed by the method of Ridgway *et al.* (1973).

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

TABLE E1
Organ Weights for F344/N Rats in the 14-Day Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride[a]

Organ	0 ppm	600 ppm	1,250 ppm	2,500 ppm	5,000 ppm[b]	7,500 ppm[c]
Male						
Necropsy body wt	218 ± 4.8	218 ± 3.2	195 ± 2.9*	105 ± 8.3**	85 ± 3.9**	
Liver	9.02 ± 0.18	12.06 ± 0.29	11.81 ± 0.17	6.08 ± 0.79	3.79 ± 0.51	
Brain	1.74 ± 0.02	1.74 ± 0.02	1.69 ± 0.02	1.66 ± 0.02*	1.61 ± 0.02**	
Heart	0.69 ± 0.02	0.67 ± 0.03	0.60 ± 0.01*	0.36 ± 0.01**	0.34 ± 0.00**	
Lungs	0.94 ± 0.02	0.98 ± 0.02	0.90 ± 0.03	0.65 ± 0.02**	0.61 ± 0.01**	
Right kidney	0.82 ± 0.03	0.87 ± 0.01	0.82 ± 0.02	0.60 ± 0.01*	0.50 ± 0.01**	
Right testis	1.12 ± 0.00	1.11 ± 0.01	1.10 ± 0.05	0.79 ± 0.04**	0.70 ± 0.03**	
Thymus	0.44 ± 0.02	0.49 ± 0.08	0.36 ± 0.03	0.06 ± 0.01**	0.03 ± 0.01**	
Female						
Necropsy body wt	153 ± 2.6	143 ± 2.1*	132 ± 0.7**	112 ± 1.3**	60 ± 1.0**	61 ± 2.2**
Liver	5.73 ± 0.21	6.66 ± 0.18	6.24 ± 0.09	6.29 ± 0.14	2.02 ± 0.10	1.80 ± 0.50
Brain	1.60 ± 0.02	1.64 ± 0.01	1.61 ± 0.01	1.56 ± 0.01	1.49 ± 0.02*	1.48 ± 0.03*
Heart	0.49 ± 0.02	0.48 ± 0.01	0.44 ± 0.01	0.39 ± 0.01**	0.26 ± 0.00**	0.25 ± 0.01**
Lungs	0.74 ± 0.02	0.78 ± 0.01	0.73 ± 0.02	0.64 ± 0.01*	0.48 ± 0.02**	0.50 ± 0.02**
Right kidney	0.57 ± 0.01	0.57 ± 0.01	0.57 ± 0.00	0.55 ± 0.01	0.37 ± 0.01**	0.49 ± 0.12*
Thymus	0.37 ± 0.03	0.33 ± 0.02	0.32 ± 0.02	0.26 ± 0.01**	0.03 ± 0.01**	0.02 ± 0.01

* Significantly different (P≤0.05) from the control group by Shirley's test (Shirley, 1977)
** P≤0.01
[a] Mean ± standard error in grams for groups of 5 animals, unless otherwise specified.
[b] There were 4/5 male and 5/5 female survivors in the 5,000 ppm group.
[c] There were 0/5 male and 4/5 female survivors in the 7,500 ppm group. There are no organ weight data for males in the 7,500 ppm group since no rats in this group survived to termination.

TABLE E2
Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 14-Day Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride[a]

Ratio (%)	0 ppm	600 ppm	1,250 ppm	2,500 ppm	5,000 ppm[b]	7,500 ppm[c]
Male						
Necropsy body wt	218 ± 4.8	218 ± 3.2	195 ± 2.9*	105 ± 8.3**	85 ± 3.9**	
Liver	41.5 ± 0.43	55.3 ± 0.62	60.6 ± 0.24**	56.8 ± 3.21	42.6 ± 3.73	
Brain	8.03 ± 0.13	8.01 ± 0.16	8.69 ± 0.07**	16.15 ± 1.26**	18.37 ± 0.61**	
Heart	3.19 ± 0.08	3.09 ± 0.09	3.07 ± 0.05	3.47 ± 0.17	3.82 ± 0.14*	
Lungs	4.31 ± 0.06	4.50 ± 0.09	4.60 ± 0.09	6.32 ± 0.40**	6.93 ± 0.23**	
Right kidney	3.78 ± 0.04	4.01 ± 0.03*	4.22 ± 0.11**	5.81 ± 0.38**	5.73 ± 0.23**	
Right testis	5.15 ± 0.11	5.12 ± 0.13	5.64 ± 0.24	7.59 ± 0.35**	8.01 ± 0.20**	
Thymus	2.04 ± 0.12	2.23 ± 0.32	1.87 ± 0.14	0.60 ± 0.11**	0.35 ± 0.08**	
Female						
Necropsy body wt	153 ± 2.6	143 ± 2.1*	132 ± 0.7**	112 ± 1.3**	60 ± 1.0**	61 ± 2.2**
Liver	37.5 ± 0.86	46.6 ± 0.85	47.3 ± 0.55	56.3 ± 0.80*	33.6 ± 1.19	28.7 ± 7.67
Brain	10.5 ± 0.15	11.5 ± 0.14**	12.2 ± 0.11**	14.0 ± 0.10**	24.9 ± 0.46**	23.7 ± 0.61**
Heart	3.19 ± 0.12	3.36 ± 0.09	3.33 ± 0.05	3.51 ± 0.08*	4.37 ± 0.07**	3.99 ± 0.14**
Lungs	4.87 ± 0.12	5.46 ± 0.05**	5.53 ± 0.13**	5.70 ± 0.05**	7.98 ± 0.29**	7.96 ± 0.25**
Right kidney	3.70 ± 0.07	3.96 ± 0.02**	4.30 ± 0.03**	4.93 ± 0.07	6.24 ± 0.20**	7.99 ± 2.15**
Thymus	2.43 ± 0.16	2.29 ± 0.12	2.44 ± 0.11	2.32 ± 0.09	0.52 ± 0.11**	0.39 ± 0.11**

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error in mg/g for groups of 5 animals, unless otherwise specified.
[b] There were 4/5 male and 5/5 female survivors in the 5,000 ppm group.
[c] There were 0/5 male and 4/5 female survivors in the 7,500 ppm group. There are no organ weight data for males in the 7,500 ppm group since no rats in this group survived to termination.

TABLE E3
Organ Weights for F344/N Rats in the 13-Week Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride[a]

Organ	0 ppm	300 ppm	500 ppm	1,000 ppm	2,000 ppm[b]
Male					
Necropsy body wt	350 ± 7.41	295 ± 4.93**	302 ± 3.96**	295 ± 4.32**	187 ± 13.43**
Liver	9.78 ± 0.50	10.21 ± 0.27	10.83 ± 0.33	11.06 ± 0.18	6.75 ± 0.68
Brain	1.97 ± 0.02	1.93 ± 0.02	1.91 ± 0.02	1.93 ± 0.02	1.77 ± 0.02**
Heart	0.95 ± 0.02	0.86 ± 0.02*	0.92 ± 0.02	0.92 ± 0.03	0.64 ± 0.04**
Lungs	1.22 ± 0.03	1.14 ± 0.03[c]	1.16 ± 0.02[c]	1.13 ± 0.02	0.89 ± 0.02[d]**
Right kidney	1.04 ± 0.01	0.97 ± 0.02	1.02 ± 0.02	1.06 ± 0.02	0.84 ± 0.04**
Right testis	1.50 ± 0.03	1.37 ± 0.03*	1.42 ± 0.01	1.47 ± 0.02	1.30 ± 0.05*
Thymus	0.27 ± 0.01	0.19 ± 0.01**	0.23 ± 0.02**	0.23 ± 0.02*	0.12 ± 0.02**
Female					
Necropsy body wt	186 ± 1.42	172 ± 1.90*	172 ± 2.35**	165 ± 1.79**	116 ± 7.52**
Liver	4.74 ± 0.06	4.93 ± 0.07	5.08 ± 0.09*	5.11 ± 0.08*	4.00 ± 0.24
Brain	1.80 ± 0.01	1.77 ± 0.02	1.77 ± 0.01	1.77 ± 0.01*	1.67 ± 0.01**
Heart	0.64 ± 0.01	0.58 ± 0.01**	0.59 ± 0.01**	0.55 ± 0.01**	0.39 ± 0.03**
Lungs	0.90 ± 0.02	0.89 ± 0.02	0.85 ± 0.02	0.82 ± 0.01**	0.68 ± 0.02**
Right kidney	0.59 ± 0.01	0.62 ± 0.01	0.66 ± 0.02*	0.64 ± 0.01*	0.59 ± 0.01
Thymus	0.22 ± 0.01	0.18 ± 0.01**	0.17 ± 0.01**	0.17 ± 0.01**	0.06 ± 0.01**.

* Significantly different (P≤0.05) from the control group by Dunn's (1964) or Shirley's (1977) test
** P≤0.01
[a] Mean ± standard error in grams for groups of 10 animals, unless otherwise specified.
[b] There were 6/10 male and 7/10 female survivors in the 2,000 ppm group.
[c] Nine animals were weighed.
[d] Five animals were weighed.

TABLE E4
Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride[a]

Ratio (%)	0 ppm	300 ppm	500 ppm	1,000 ppm	2,000 ppm[b]
Male					
Necropsy body wt	350 ± 7.41	295 ± 4.93**	302 ± 3.96**	295 ± 4.32**	187 ± 13.43**
Liver	27.9 ± 1.19	34.6 ± 0.70**	35.9 ± 0.76**	37.5 ± 0.37**	35.9 ± 1.89**
Brain	5.64 ± 0.11	6.56 ± 0.05**	6.35 ± 0.11**	6.54 ± 0.09**	9.74 ± 0.74**
Heart	2.70 ± 0.04	2.92 ± 0.05**	3.05 ± 0.08**	3.11 ± 0.08**	3.41 ± 0.08**
Lungs	3.49 ± 0.05	3.85 ± 0.05**[c]	3.87 ± 0.07**[c]	3.85 ± 0.09**	4.94 ± 0.37[d]**
Right kidney	2.97 ± 0.03	3.30 ± 0.05**	3.40 ± 0.07**	3.60 ± 0.10**	4.59 ± 0.31**
Right testis	4.28 ± 0.07	4.65 ± 0.05**	4.73 ± 0.08**	5.00 ± 0.10**	7.11 ± 0.35**
Thymus	0.77 ± 0.03	0.66 ± 0.03	0.76 ± 0.06	0.79 ± 0.06	0.61 ± 0.11
Female					
Necropsy body wt	186 ± 1.4	172 ± 1.9**	172 ± 2.4**	165 ± 1.8**	116 ± 7.5**
Liver	25.5 ± 0.33	28.7 ± 0.28**	29.5 ± 0.29**	31.0 ± 0.43**	34.8 ± 0.92**
Brain	9.71 ± 0.06	10.28 ± 0.10**	10.30 ± 0.09**	10.73 ± 0.13**	14.82 ± 1.00**
Heart	3.43 ± 0.07	3.38 ± 0.04	3.42 ± 0.08	3.32 ± 0.07	3.39 ± 0.08
Lungs	4.84 ± 0.09	5.15 ± 0.09*	4.91 ± 0.06	4.98 ± 0.08	6.00 ± 0.40**
Right kidney	3.18 ± 0.05	3.59 ± 0.05**	3.83 ± 0.08**	3.91 ± 0.06**	5.26 ± 0.45**
Thymus	1.19 ± 0.05	1.03 ± 0.04*	1.01 ± 0.04**	1.04 ± 0.05*	0.47 ± 0.07**

* 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 in mg/g for groups of 10 animals, unless otherwise specified.
[b] There were 6/10 male and 7/10 female survivors in the 2,000 ppm group.
[c] Nine animals were weighed.
[d] Five animals were weighed.

TABLE E5
Organ Weights for F344/N Rats in the 9-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride[a]

Organ	0 ppm	150 ppm
Male		
Necropsy body wt	433.6 ± 4.1	345.0 ± 8.1**
Brain	2.02 ± 0.02	1.99 ± 0.02
Kidney	2.48 ± 0.03	3.40 ± 0.21**
Liver	10.29 ± 0.15	19.51 ± 0.55**
Female		
Necropsy body wt	237.6 ± 5.4	197.2 ± 2.9**
Brain	1.85 ± 0.01	1.83 ± 0.01
Kidney	1.39 ± 0.02	1.59 ± 0.03**
Liver	5.75 ± 0.11	8.80 ± 0.41**

** Significantly different ($P \leq 0.01$) from the control group by Wilcoxon's test (Hollander and Wolfe, 1973)
[a] Mean ± standard error in grams for groups of 10 animals

TABLE E6
Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 9-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride[a]

Ratio (%)	0 ppm	150 ppm
Male		
Necropsy body wt	433.6 ± 4.1	345.0 ± 8.1**
Brain	4.66 ± 0.071	5.78 ± 0.13**
Kidney	5.71 ± 0.070	9.85 ± 0.89**
Liver	23.7 ± 0.37	56.6 ± 2.33**
Female		
Necropsy body wt	237.6 ± 5.4	197.2 ± 2.9**
Brain	7.79 ± 0.15	9.30 ± 0.012**
Kidney	5.84 ± 0.11	8.08 ± 0.20**
Liver	24.2 ± 0.44	44.7 ± 2.25**

** Significantly different ($P \leq 0.01$) from the control group by Wilcoxon's test
[a] Mean ± standard error in mg/g for groups of 10 animals

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

3,3′-Dimethylbenzidine dihydrochloride was obtained in two lots from the Taylor Chemical Company. Lot no. T122380 was obtained on 15 December 1981, and lot no. IP22 was obtained on 21 September 1982. Purity and identity analyses were conducted at Midwest Research Institute (MRI; Kansas City, MO). MRI reports on analyses performed in support of the 3,3′-dimethylbenzidine dihydrochloride studies are on file at the National Institute of Environmental Health Sciences.

Both lots of the test chemical, a yellow microcrystalline powder, were identified as 3,3′-dimethylbenzidine dihydrochloride 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 3,3′-dimethylbenzidine dihydrochloride (Figures F1 and F2) (Sadtler Standard Spectra).

The purity of both lots was determined by elemental analysis, Karl Fischer water analysis, potentiometric titrations, and chromatographic analyses. Two potentiometric titrations were performed. Titration of the amino groups was performed in glacial acetic acid containing mercury (II) acetate with 0.1 N perchloric acid. Titration of the dihydrochloride salt was performed in water with 0.1 N sodium hydroxide. Normal phase thin-layer chromatography was performed on silica gel plates with chloroform:methyl ethyl ketone:methanol:ammonium hydroxide (50:30:19:1). Reverse phase thin-layer chromatography was performed on Whatman $KC_{18}F$ plates with methanol:water:ammonium hydroxide (80:18:2). Visualization was accomplished with visible light, short (254 nm), and long (366 nm) wavelength ultraviolet light, iodine vapor, and by 0.5% ninhydrin in *n*-butanol, followed by heating at 110° C for 5 to 10 minutes. High-performance liquid chromatography was performed with a μBondapak C_{18} column and a mobile phase of 78% 5 mM aqueous sodium heptanesulfonate containing 1% (v/v) acetic acid and 22% 5 mM methanolic sodium heptanesulfonate containing 1% (v/v) acetic acid at a flow rate of 1 mL/min. Ultraviolet detection was at 254 nm.

For lot no. T122380, elemental analysis for carbon, hydrogen, chlorine, and nitrogen was in agreement with the theoretical values. Karl Fischer analysis of this lot indicated the presence of 0.10% water. Titration of the amino groups indicated a purity of 99.5%. Titration of the dihydrochloride salt indicated a purity of 100.9%. Normal phase thin-layer chromatography indicated a major product spot and a single trace impurity. Reverse phase thin-layer chromatography indicated a major product spot and two trace impurities. High-performance liquid chromatography of this lot indicated two impurities with a combined peak area of 0.32% relative to that of the major peak.

For lot no. IP22, elemental analysis for carbon, hydrogen, chlorine, and nitrogen was in agreement with the theoretical values. Karl Fischer analysis indicated the presence of 0.07% water. Titration of the amino groups indicated a purity of 99.0%. Titration of the dihydrochloride salt indicated a purity of 99.7%. Normal phase thin-layer chromatography indicated a major product spot and a single trace impurity. Reverse phase thin-layer chromatography indicated a major product spot and a single trace impurity. High-performance liquid chromatography of this lot indicated no impurities greater than 0.1% relative to the major peak area.

Stability studies performed by high-performance liquid chromatography with the system described above but with a solvent ratio of 70:30 and with acetanilide added as an internal standard indicated that 3,3′-dimethylbenzidine dihydrochloride, when protected from light, was stable as a bulk chemical for at least 2 weeks at temperatures up to 60° C. During the 14-month studies, the stability of the bulk

chemical was monitored by high-performance liquid chromatography and nonaqueous amine titration; no degradation of the study material was seen throughout the studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Initially, the dose formulations were prepared by mixing appropriate quantities of 3,3′-dimethylbenzidine dihydrochloride with feed in a blender (Patterson-Kelley Twin Shell). Stability studies were conducted at the analytical laboratory. Dose formulations were extracted with a solution consisting of 5 N sodium hydroxide:methanol (0.05:1). The extracts were combined and extracted with 0.5 N hydrochloric acid. These extracts were washed with cyclohexane, pH adjusted with 10 N sodium hydroxide, and extracted with methylene chloride. The methylene chloride extract was centrifuged, p-terphenyl was added as an internal standard, and 3,3′-dimethylbenzidine was determined by gas chromatography performed with flame ionization detection, using a 3% OV-17 on 100/120 Supelcoport column, with nitrogen as the carrier at 30 mL/minute.

3,3′-Dimethylbenzidine dihydrochloride in rodent feed (NIH 07 Rat and Mouse Ration) at the 675 ppm dose level was found to be unstable when stored at or above 5° C. Dose formulations stored under simulated animal cage conditions (open to light and air) lost approximately 18% and 21% of chemical after 3 and 7 days, respectively. Losses of 14.5% and 23.4% were seen in samples stored in sealed glass bottles at 5° C and at room temperature, respectively, for 2 weeks. Although no significant loss was observed after 7-day storage at -20° C, a statistically significant loss (4.8%) was detected after 15 days storage at -20° C. A minimum of 2.9% loss of chemical was required to conclude that the dose formulation was unstable (95% confidence level).

Because of the instability of the test chemical in feed, new dose formulations were prepared by mixing appropriate quantities of 3,3′-dimethylbenzidine dihydrochloride and tap water (for 14-day studies) or distilled water (for 13-week or 14-month studies) to give the required concentrations (w/v) (Table F1). Stability studies were performed at the analytical laboratory with high-performance liquid chromatography using a μBondapak C_{18} column and a mobile phase of water:methanol (55:45) containing 0.06 N sodium bromide and propiophenone as an internal standard. Ultraviolet detection was at 280 nm.

3,3′-Dimethylbenzidine dihydrochloride in water at the 675 ppm dose level was found to be stable for up to 15 days when stored protected from light in sealed containers at room temperature. Storage under simulated animal cage conditions (open to air and light) for 48 hours had no measurable effect on chemical stability. Therefore, drinking water was selected as the vehicle of choice for administration of the chemical to the study animals.

Periodic analyses of the dose formulations of 3,3′-dimethylbenzidine dihydrochloride 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. For the 13-week studies, dose formulations were analyzed twice prior to study initiation, at the midpoint of the study, and again at the end of the study (Table F2). During the 14-month studies, the formulations used for dosing were analyzed by ultraviolet spectroscopy at a minimum of once every 4 weeks. Because 64/77 formulations were within the specified ±10% of the target concentrations, it was estimated that 83% of the formulations were prepared within specifications. Results of the dose formulation analyses for the chronic studies are presented in Table F3. Results of periodic referee analysis performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table F4).

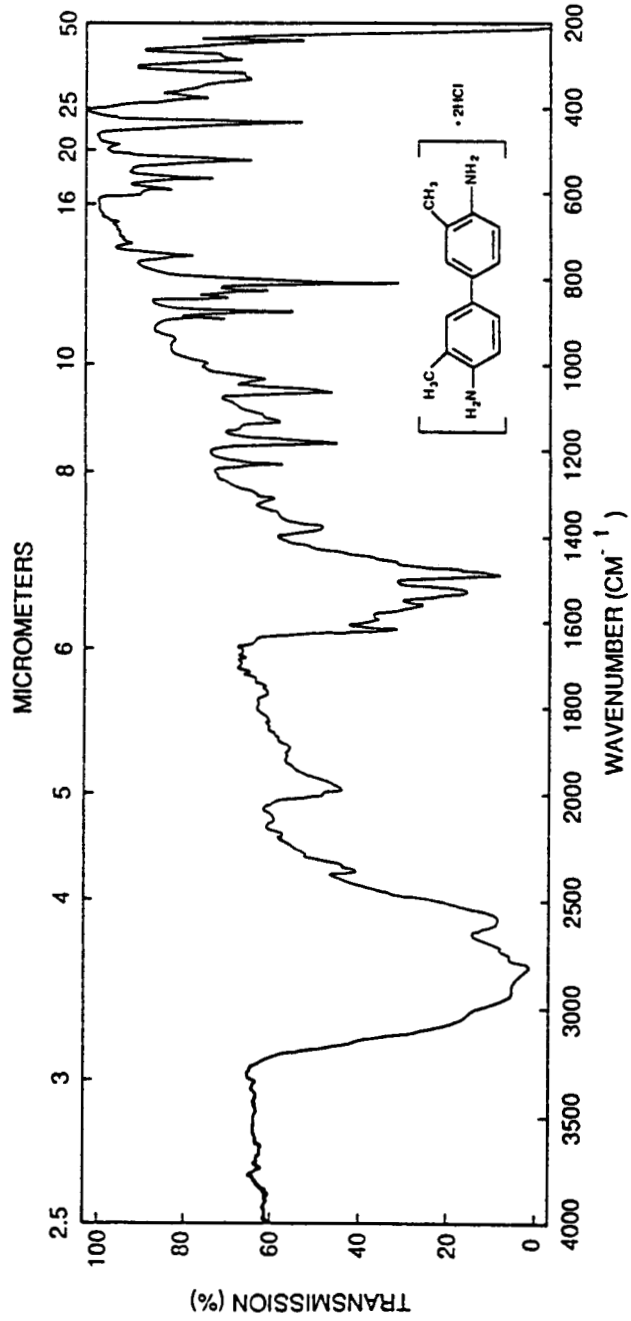

FIGURE F1
Infrared Absorption Spectrum of 3,3′-Dimethylbenzidine Dihydrochloride

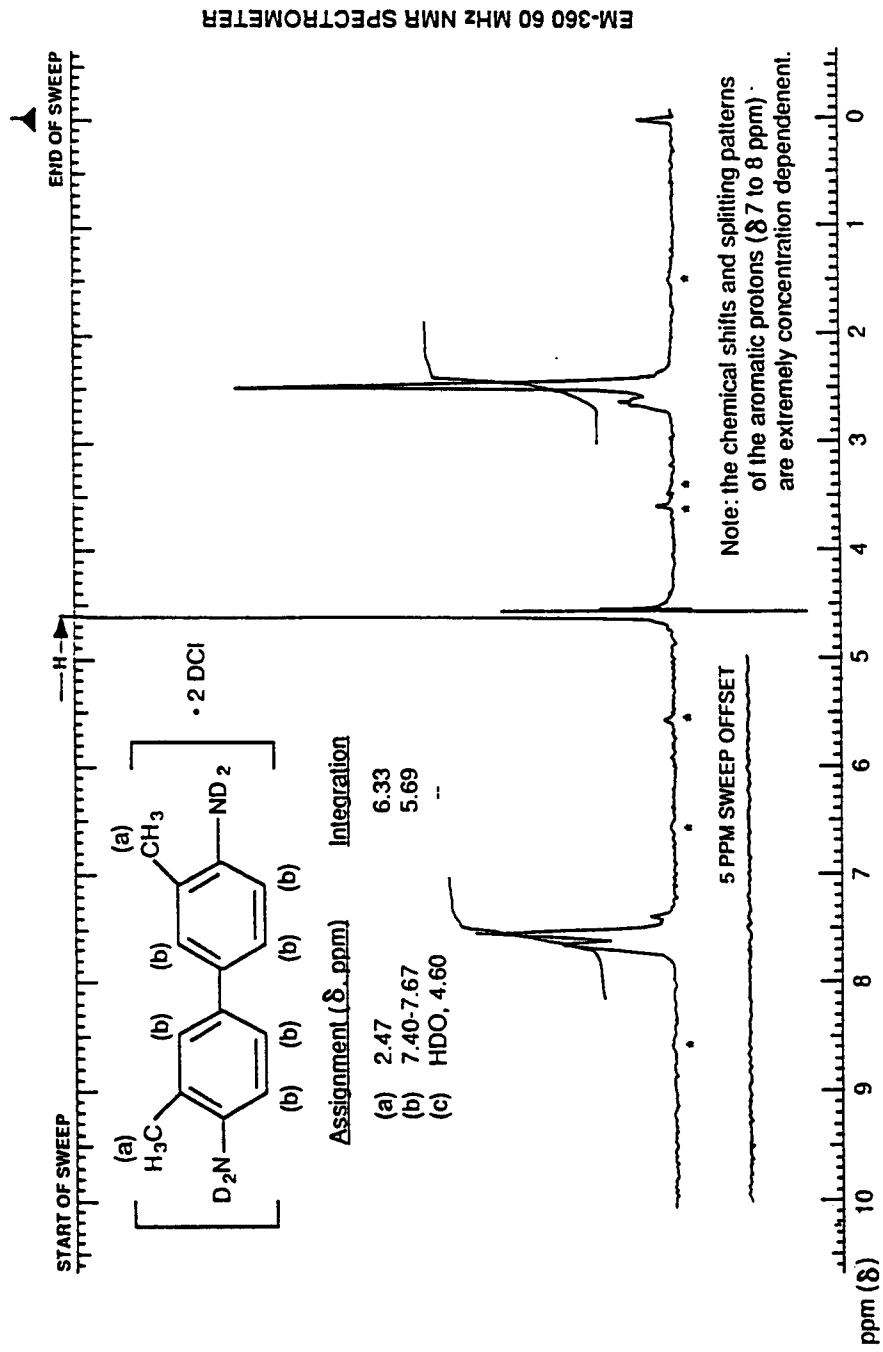

FIGURE F2
Nuclear Magnetic Resonance Spectrum of 3,3′-Dimethylbenzidine Dihydrochloride

TABLE F1
Preparation and Storage of Dose Formulations in the Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

14-Day Studies	13-Week Studies	14-Month Studies
Preparation		
Weighed amount of 3,3'-dimethylbenzidine dihydrochloride 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.	Weighed amount of 3,3'-dimethylbenzidine dihydrochloride 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.	Same as 13-week studies
Chemical Lot Number		
T122380	T122380	IP22
Maximum Storage Time		
3.5 days	Same as 14-day studies	1 week
Storage Conditions		
In the dark at room temperature	Same as 14-day studies	Same as 14-day studies

TABLE F2
Results of Analysis of Dose Formulations in the 13-Week Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

Date Mixed	Concentration of 3,3′-Dimethylbenzidine Dihydrochloride in Drinking Water (ppm)		Determined as a Percent of Target
	Target	Determined[a]	
07/23/82[b]	300	330	110.0
	500	530	106.0
	1,000	1,030	103.0
	2,000	2,020	101.0
	4,000	4,130	103.3
07/29/82[c]	500	490	98.0
07/30/82[d]	300	300	100.0
	500	490	98.0
	1,000	960	96.0
	2,000	1,950	97.5
	4,000	3,890	97.3
09/10/82	300	300	100.0
	500	490	98.0
	1,000	960	96.0
	2,000	2,000	100.0
09/10/82[d]	300	290	96.7
	500	490	98.0
	1,000	980	98.0
	2,000	2,080	104.0
10/29/82	300	300	100.0
	500	500	100.0
	1,000	1,010	101.0
	2,000	2,090	105.0

[a] Results of duplicate analysis
[b] One week before start of study
[c] One day before start of study; remixed in distilled water
[d] Animal room samples

TABLE F3
Results of Analysis of Dose Formulations in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

Date Mixed	Determined Concentration of 3,3′-Dimethylbenzidine Dihydrochloride in Water for Target Concentration (ppm)[a]				
	30	70	70	150	150
06/23/83	32	74	66	152	147
07/18/83	30	70	58[b]	150	139
07/18/83			57[b,c]		
07/21/83			71[c]		
08/15/83	33	72	62[b]	153	145
08/15/83			61[b]		
08/19/83			70[c]		
09/12/83	34[b]	72	124[b]	149	151
09/14/83	29[b]		62[b,c]		
09/15/83	29[c]		68[c]		
10/10/83	29	69	68	147	138
11/07/83	34[b]	70	60[b]	149	149
11/11/83	31[c]		71[c]		
12/05/83	30	71	68	151	149
01/02/84	27[b]	71	63[c]	150	141
01/09/84	28[c]		61[d]		
01/30/84	39[b,e]	73	75	160	161
02/02/84	29[c]				
02/27/84	27	67	63	147	140
03/26/84	30	71	70	150	135
04/23/84	30	69	70	150	
05/21/84	28	81[b]	87[b]	151	
05/25/84		68[c]	65[c]		
06/18/84	28	67	74	146	
07/16/84	28	69	65	148	
08/14/84	29	69	63	149	
Mean (ppm)	31	71	70	150	145
Standard deviation	3.14	3.32	15.54	3.2	7.44
Coefficient of variation (percent)	10.3	4.7	22.1	2.1	5.1
Range (ppm)	27-39	67-81	58-124	146-160	135-161
Number of samples	17	16	17	16	11

[a] Results of duplicate analysis
[b] Out of specifications; not used in study
[c] Remix; not included in the mean
[d] No remix done
[e] Sample rediluted for confirmation; results comparable (39 ppm)

TABLE F4
Results of Referee Analysis of Dose Formulations in the 14-Month Drinking Water Studies of 3,3'-Dimethylbenzidine Dihydrochloride

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory[a]	Referee Laboratory[b]
06/23/83	70	74	69.8
12/05/83	150	151	152
06/18/84	30	27.6	28.2

[a] Results of duplicate analysis
[b] Results of triplicate analysis

APPENDIX G
WATER AND COMPOUND CONSUMPTION BY RATS IN THE 14-MONTH DRINKING WATER STUDIES

TABLE G1
Water and Compound Consumption by Male F344/N Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride

	0 ppm		30 ppm			70 ppm			150 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	26.6	235.6	23.4	229.5	3.1	21.0	232.7	6.3	19.7	221.0	13.4
5	23.7	261.8	22.3	254.3	2.6	21.4	254.9	5.9	19.2	239.0	12.1
8	24.3	304.8	22.7	301.2	2.3	19.3	304.0	4.5	19.6	276.6	10.6
9	23.0	320.2	21.9	316.0	2.1	21.6	316.3	4.8	16.6	286.7	8.7
12	23.2	352.1	21.2	345.0	1.8	19.6	339.8	4.0	16.8	314.2	8.0
13	20.5	354.3	22.6	350.6	1.9	21.0	345.6	4.3	16.7	317.4	7.9
17	24.9	380.2	24.5	375.8	2.0	21.3	371.8	4.0	17.3	336.7	7.7
21	22.5	401.0	20.9	399.4	1.6	19.4	387.5	3.5	17.1	352.0	7.3
25	22.7	412.4	20.6	410.2	1.5	19.4	401.1	3.4	17.4	361.6	7.2
29	22.6	422.7	20.2	412.8	1.5	20.4	404.6	3.5	19.0	361.3	7.9
33	22.6	435.8	21.1	426.1	1.5	20.2	418.4	3.4	21.0	367.7	8.6
37	21.8	447.4	21.3	432.4	1.5	20.0	426.2	3.3	23.6	367.7	9.6
41	21.7	447.3	19.2	439.8	1.3	20.2	423.2	3.3	29.2	356.0	12.3
45	22.7	458.5	19.6	448.6	1.3	20.5	429.5	3.3	37.8	341.5	16.6
49	19.7	454.2	19.7	443.3	1.3	20.5	431.8	3.3	40.8	319.0	19.2
53	20.0	453.0	21.1	443.1	1.4	20.7	423.3	3.4	47.2	331.9	21.3
57	20.3	446.5	20.9	432.0	1.5	21.9	408.4	3.7			
Mean for Weeks											
1-13	23.5	304.8	22.3	299.4	2.3	20.7	298.9	5.0	18.1	275.8	10.1
14-52	22.3	428.8	20.8	420.9	1.5	20.2	410.5	3.5	24.8	351.5	10.7
>52	20.1	449.8	21.0	437.6	1.4	21.3	415.9	3.6	47.2	331.9	21.3

[a] Grams of water consumed per animal per day; not corrected for wastage.
[b] Estimated milligrams of 3,3′-dimethylbenzidine dihydrochloride consumed per day per kilogram of body weight

TABLE G2
Water and Compound Consumption by Female F344/N Rats in the 14-Month Drinking Water Study of 3,3′-Dimethylbenzidine Dihydrochloride

Week	0 ppm		30 ppm			70 ppm			150 ppm		
	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	22.6	154.3	23.8	152.3	4.7	23.9	148.7	11.3	23.4	145.6	24.1
5	23.3	163.6	20.2	159.6	3.8	20.6	156.3	9.2	16.2	152.9	15.9
8	21.0	183.9	28.1	176.4	4.8	22.0	173.6	8.9	18.2	168.3	16.2
9	18.3	188.2	22.1	183.3	3.6	18.3	176.2	7.3	13.5	172.8	11.7
12	26.9	198.1	29.4	191.4	4.6	21.8	185.2	8.2	19.9	181.7	16.4
13	24.1	199.4	23.2	194.3	3.6	21.1	187.2	7.9	15.1	181.8	12.5
17	27.5	210.2	25.2	204.5	3.7	20.1	196.2	7.2	14.4	189.5	11.4
21	20.0	217.2	19.4	210.9	2.8	16.4	203.2	5.7	13.4	196.1	10.3
25	18.2	224.6	17.3	217.9	2.4	15.8	208.9	5.3	12.8	199.8	9.6
29	21.0	228.1	19.6	222.2	2.6	22.4	212.2	7.4	13.5	202.7	10.0
33	18.9	238.1	20.1	227.7	2.6	16.7	218.4	5.3	13.4	206.4	9.7
37	20.7	247.0	17.4	235.9	2.2	18.1	219.7	5.8	12.6	204.7	9.2
41	17.7	250.9	17.6	242.8	2.2	17.3	222.9	5.4	14.4	210.5	10.3
45	17.4	263.8	16.5	252.0	2.0	17.5	222.8	5.5	13.9	209.5	10.0
49	15.5	270.7	16.8	262.8	1.9	18.5	227.7	5.7	15.4	210.6	11.0
53	16.3	279.6	15.7	267.8	1.8	18.3	228.1	5.6	21.1	208.6	15.1
57	14.9	287.2	16.3	270.0	1.8	20.2	232.2	6.1	22.9	213.8	16.1
Mean for Weeks											
1-13	22.7	181.3	24.4	176.2	4.2	21.3	171.2	8.8	17.7	167.2	16.1
14-52	19.7	239.0	18.9	230.7	2.5	18.1	214.7	5.9	13.8	203.3	10.2
>52	15.6	283.4	16.0	268.9	1.8	19.2	230.2	5.8	22.0	211.2	15.6

[a] Grams of water consumed per animal per day; not corrected for wastage.
[b] Estimated milligrams of 3,3′-dimethylbenzidine dihydrochloride consumed per day per kilogram of body weight

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

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

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

[a] NCI, 1976; NIH, 1978
[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)	22.85 ± 0.98	21.3–24.9	15
Crude fat (% by weight)	5.14 ± 0.66	3.3–6.3	15
Crude fiber (% by weight)	3.49 ± 0.32	2.8–3.8	15
Ash (% by weight)	6.73 ± 0.32	6.3–7.3	15
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	0.655–1.050	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	1.830–2.520	5
Linolenic	0.258 ± 0.040	0.210–0.308	5
Vitamins			
Vitamin A (IU/kg)	13,160 ± 5,475	4,100–24,000	15
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)	19.40 ± 3.83	12.0–27.0	15
Riboflavin (ppm)	7.60 ± 0.85	6.10–8.20	5
Niacin (ppm)	97.80 ± 31.68	65.0–150.0	5
Pantothenic acid (ppm)	30.06 ± 4.31	23.0–34.0	5
Pyridoxine (ppm)	7.68 ± 1.31	5.60–8.80	5
Folic acid (ppm)	2.62 ± 0.89	1.80–3.70	5
Biotin (ppm)	0.254 ± 0.053	0.19–0.32	5
Vitamin B_{12} (ppb)	24.21 ± 12.66	10.6–38.0	5
Choline (ppm)	3,122 ± 416.8	2,400–3,430	5
Minerals			
Calcium (%)	1.30 ± 0.13	1.12–1.54	15
Phosphorus (%)	0.97 ± 0.07	0.87–1.10	15
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	Range	Number of Samples
Arsenic (ppm)	0.52 ± 0.19	0.17–0.77	15
Cadmium (ppm)[a]	<0.10		15
Lead (ppm)	0.62 ± 0.24	0.33–1.27	15
Mercury (ppm)[a]	<0.05		15
Selenium (ppm)	0.35 ± 0.05	0.28–0.42	15
Aflatoxins (ppb)[a]	<5.0		15
Nitrate nitrogen (ppm)	8.32 ± 3.92	0.10–15.0	15
Nitrite nitrogen (ppm)	1.13 ± 2.04	0.10–7.20	15
BHA (ppm)[b]	2.20 ± 0.77	2.00–5.05	15
BHT (ppm)[b]	2.13 ± 1.19	1.00–4.00	15
Aerobic plate count (CFU/g)[c]	50,480 ± 39,406	7,400–130,000	15
Coliform (MPN/g)[d]	42.93 ± 116.45	3.00–460	15
E. coli (MPN/g)[d]	3.00		15
Total nitrosamines (ppb)[e]	5.89 ± 7.12	1.80–30.90	15
N-Nitrosodimethylamine (ppb)[e]	4.89 ± 7.14	0.80–30.00	15
N-Nitrosopyrrolidine (ppb)[e]	1.00 ± 0.17	0.90–1.50	15
Pesticides (ppm)			
α-BHC[a,f]	<0.01		15
β-BHC[a,f]	<0.02		15
γ-BHC[a,f]	<0.01		15
δ-BHC[a,f]	<0.01		15
Heptachlor[a]	<0.01		15
Aldrin[a]	<0.01		15
Heptachlor epoxide[a]	<0.01		15
DDE[a]	<0.01		15
DDD[a]	<0.01		15
DDT[a]	<0.01		15
HCB[a]	<0.01		15
Mirex[a]	<0.01		15
Methoxychlor[a]	<0.05		15
Dieldrin[a]	<0.01		15
Endrin[a]	<0.01		15
Telodrin[a]	<0.01		15
Chlordane[a]	<0.05		15
Toxaphene[a]	<0.1		15
Estimated PCBs[a]	<0.2		15
Ronnel[a]	<0.01		15
Ethion[a]	<0.02		15
Trithion[a]	<0.05		15
Diazinon[a]	<0.1		15
Methyl parathion[a]	<0.02		15
Ethyl parathion[a]	<0.02		15
Malathion[g]	0.14 ± 0.11	0.05–0.45	15
Endosulfan I[a]	<0.01		15
Endosulfan II[a]	<0.01		15
Endosulfan sulfate[a]	<0.03		15

[a] All values were less than the detection limit, given in the table as the mean.
[b] Sources of contamination: soy oil and fish meal
[c] CFU = colony-forming unit
[d] MPN = most probable number
[e] All values were corrected for percent recovery.
[f] BHC = hexachlorocyclohexane or benzene hexachloride
[g] Ten lots contained more than 0.05ppm

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 13-week and 14-month 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:	Preinitiation and termination of 13-week study.
RCV (rat coronavirus)	
Sendai	
ELISA:	Initiation, 6 months, 12 months, and
RCV/SDA (sialodacryoadentis virus)	termination of 14-month study.
Hemagglutination Inhibition:	Preinitiation and termination of 13-week study;
PVM (pneumonia virus of mice)	initiation, 6 months, 12 months, and
KRV (Kilham rat virus)	termination of 14-month study.
H-1 (Toolan's H-1 virus)	
Sendai	Initiation, 6 months, 12 months, and termination of 14-month study.

TABLE I1
Murine Virus Antibody Determinations for Rats in the 14-Month Drinking Water Studies of 3,3′-Dimethylbenzidine Dihydrochloride

	Interval	Number of Animals	Positive Serologic Reaction for
13-Week Studies	0	10/10	none
	13 weeks	10/10	none
14-Month Studies	0	10/10	none
	6 months	10/10	none
	12 months	1/10	KRV
	14 months	1/10	KRV

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PRINTED AS OF APRIL 1991

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	1,3-Dichloropropane (Telone II®)
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

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
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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
386	Tetranitromethane
393	Sodium Fluoride

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