NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 412

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 4,4′-DIAMINO-2,2′-STILBENEDISULFONIC ACID, DISODIUM SALT

(CAS NO. 7336-20-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

FOREWORD

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

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

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

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

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

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P.O. Box 12233
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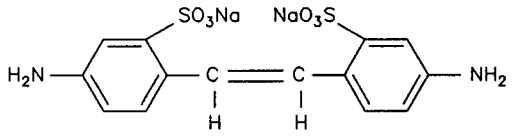
ABSTRACT



4,4′-DIAMINO-2,2′-STILBENEDISULFONIC ACID, DISODIUM SALT

CAS No. 7336-20-1

Chemical Formula: $C_{14}H_{12}N_2O_6S_2 \cdot 2Na$ Molecular Weight: 414.42

Synonyms: Amsonic acid; diaminostilbene disulphonate (DASD); 2,2′-(1,2-ethenediyl)bis[5-amino-benzenesulfonic acid]; 2,2′-disulfo-4,4′-stilbenediamine; 2,2′-stilbenedisulfonic acid; 4,4′-diamino-2,2′-benzenesulfonic acid; 2,2′-(1,2-ethenediyl)bis(5-amino-) diaminostilbenedisulfonic acid; flavonic acid; *p,p′*-diaminostilbene-*o,o′*-disulfonic acid; 4,4′-diaminostilbene-2,2′-disulfonic acid

4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, is used in the synthesis of dyes and optical brighteners or fluorescent whitening agents. Toxicology and carcinogenesis studies were conducted by administering the chemical (approximately 14% water, 6% sodium chloride, 4% impurities, and 76% 4,4′-diamino-2,2′-stilbenedisulfonic acid) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary cells.

14-Day Studies: Groups of five rats and five mice of each sex were given 0, 6,250, 12,500, 25,000, 50,000, or 100,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed for 14 days. All rats and mice survived to the end of the studies. The mean body weight gain of male rats receiving 50,000 or 100,000 ppm and of female rats and male and female mice receiving 100,000 ppm was significantly lower than those of the respective controls. Clinical findings included diarrhea in the rats and mice receiving 100,000 ppm. There were no chemical-related changes in absolute or relative organ weights in rats or mice. There were no gross or microscopic lesions related to chemical administration in rats or mice.

13-Week Studies: Groups of 10 rats and 10 mice of each sex were given 0, 6,250, 12,500, 25,000, 50,000, or 100,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed for 13 weeks. One female rat, six male mice, and one female mouse in the 100,000 ppm dose groups died during the studies. Mean body weight gain was significantly decreased in male rats and female mice receiving 50,000 or 100,000 ppm, in male mice receiving 25,000, 50,000, or 100,000 ppm, and in female rats receiving 100,000 ppm. Clinical findings in rats that received 50,000 or 100,000 ppm and in mice that received 100,000 ppm included diarrhea, emaciation, and hyperemia of the perineum. There were no biologically significant changes in absolute or relative organ weights or clinical pathology results in rats or mice. Histopathologic lesions present in rats receiving 100,000 ppm were bone marrow hypercellularity and chronic inflammation of the anus and rectum. Ulcerative inflammation of the anus and rectum was observed in mice receiving 25,000 ppm and above. Female mice in the 6,250, 12,500,

25,000, and 50,000 ppm dose groups had increased incidences of cystic endometrial hyperplasia.

2-Year Studies: Doses selected for the 2-year studies were based on mortality, decreased body weight gains, and the presence of diarrhea and chronic inflammation of the anus/rectum in rats and mice during the 13-week studies. Groups of 60 rats of each sex were given 0, 12,500 or 25,000 ppm and groups of 60 mice of each sex were given 0, 6,250, or 12,500 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed for up to 103 weeks. Interim evaluations were performed on 10 rats and 10 mice from each dose group at 15 months. There were no biologically significant absolute or relative organ weight, clinical pathology, or histopathology findings in rats or mice administered 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed for 15 months.

Body Weight, Feed Consumption, Survival, and Clinical Findings in the 2-Year Studies: Mean body weights were marginally decreased for high-dose male and female rats and female mice. Feed consumption by dosed rats and mice was similar to feed consumption by the controls throughout the studies. Survival was similar among control and treated groups of rats and mice. No clinical findings related to chemical administration were observed in rats or mice.

Nonneoplastic and Neoplastic Effects in the 2-Year Studies: There were no chemical-related increased incidences of neoplasms at any site in rats. Ulcers of the forestomach or glandular stomach occurred in dosed rats (males: 1/50, 5/50, 4/50; females: 0/50, 1/50, 4/50), and may have been related to the administration of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt. There were no chemical-related incidences of neoplasms, nonneoplastic lesions, or other toxic effects in mice in the 2-year studies. Although the animals might have been able to tolerate slightly higher doses, results of the 13-week studies indicate that a doubling of the highest doses could not have been tolerated.

Genetic Toxicology: 4,4′-Diamino-2,2′-stilbenedisulfonic acid was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 with or without S9 metabolic activation. 4,4′-Diamino-2,2′-stilbenedisulfonic acid did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in male or female F344/N rats receiving 12,500 or 25,000 ppm. There was *no evidence of carcinogenic activity* of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in male or female $B6C3F_1$ mice receiving 6,250 or 12,500 ppm.

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

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
0, 12,500, or 25,000 ppm in feed	0, 12,500, or 25,000 ppm in feed	0, 6,250, or 12,500 ppm in feed	0, 6,250, or 12,500 ppm in feed
Body weights			
High-dose group marginally lower than controls	High-dose group marginally lower than controls	Dosed groups similar to controls	High-dose group marginally lower than controls
2-Year survival rates			
22/50, 20/50, 24/50	30/50, 33/50, 33/50	43/50, 40/49, 42/50	43/50, 43/50, 38/49
Nonneoplastic effects			
None	None	None	None
Neoplastic effects			
None	None	None	None
Level of evidence of carcinogenic activity			
No evidence	No evidence	No evidence	No evidence
Genetic toxicology			
Salmonella typhimurium gene mutation:	Negative with and without S9 in strains TA100, TA1535, TA1537, and TA98		
Sister chromatid exchanges			
Chinese hamster ovary cells *in vitro*:	Negative with and without S9		
Chromosomal aberrations			
Chinese hamster ovary cells *in vitro*:	Negative with and without S9		

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 cannot be evaluated because of major flaws (**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 five categories 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 is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration 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 incidence 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.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Report Review Subcommittee who evaluated the draft NTP Technical Report on 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, on July 9, 1991, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On July 9, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.R. Hailey, NIEHS, introduced the toxicology and carcinogenesis studies of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt (amsonic acid), by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on the survival and body weight effects, and commenting on compound-related nonneoplastic lesions in rats. The proposed conclusions were *no evidence of carcinogenic activity* of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in male or female F344/N rats or in male or female B6C3F$_1$ mice.

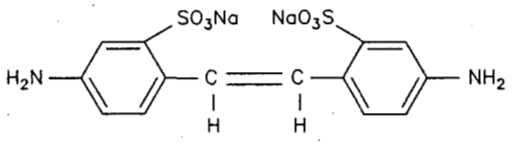
Dr. Hayden, a principal reviewer, agreed with the proposed conclusions. To emphasize the lack of toxicity, especially in mice, he thought a statement might be added to the conclusion indicating there was no evidence of toxic or nonneoplastic activity in male or female mice. Dr. Hailey said such a statement would be added to the Abstract.

Dr. Zeise, the second principal reviewer, agreed in principle with the proposed conclusions. She said that it should be noted that male and female rats may have been able to tolerate higher doses. Dr. Hailey said he agreed that females could have tolerated higher doses but considered the doses in males to be adequate. Dr. Zeise commented that the summary tables provided combined incidence data for mammary tumors (adenomas, fibroadenomas, and adenocarcinomas) indicating significantly increased levels for female rats, and that this finding should be addressed in the report. Dr. Hailey said this combination would be eliminated because the morphological continuum seen with many neoplastic processes is not seen with fibroadenomas.

Mr. Beliczky, the third principal reviewer, agreed with the proposed conclusions. He thought it would be of value for NIOSH to evaluate the facility that manufactured the amsonic acid in view of sexual dysfunction reported by workers and uterotrophic effects observed during animal studies.

Dr. Hayden moved that the Technical Report on 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity.* Mr. Beliczky seconded the motion, which was accepted unanimously with ten votes.

INTRODUCTION



4,4′-DIAMINO-2,2′-STILBENEDISULFONIC ACID, DISODIUM SALT

CAS No. 7336-20-1

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CHEMICAL AND PHYSICAL PROPERTIES, PRODUCTION, AND USE

4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, is a yellow, microcrystalline powder. It is soluble in alcohol and ether, poorly soluble in water, and forms crystalline salts with many bisquaternary ammonium bases. 4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, may be prepared from boiling the sodium salt of *p*-nitrotoluene-*o*-sulfonate in water with caustic soda followed by a reduction with zinc dust (*Condensed Chemical Dictionary*, 1977) or by catalytic reduction of 4,4′-dinitro-2,2′-stilbenedisulfonic acid.

The United States production of 4,4′-diamino-2,2′-stilbenedisulfonic acid was 7,623,000 pounds in 1975 (USITC, 1975). It is used primarily in the synthesis of dyes, such as Chrysophenine (Direct Yellow 12) or the nonethylated dye Brilliant Yellow (Direct Yellow 4), and in the synthesis of optical brighteners or fluorescent whitening agents (FWAs). In 1979, the National Institute for Occupational Safety and Health (NIOSH) estimated that 15,000 tons of fluorescent whitening agents were produced in the United States annually by 11 manufacturers. Many of these fluorescent whitening agents are synthesized from 4,4′-diamino-2,2′-stilbenedisulfonic acid and other materials. Fluorescent whitening agents are added to paper, leather, fabrics, plastics, and detergents to enhance colors and whiteness. These whitening agents have the property of absorbing ultraviolet light and reemitting high levels of blue-green light (*Kirk-Othmer*, 1983a,b).

HUMAN EXPOSURE

From a survey conducted from 1981 to 1983, NIOSH estimated that 948 workers may be exposed to the parent compound (4,4′-diamino-2,2′-stilbenedisulfonic acid) and intermediates in production facilities (NIOSH, 1991). However, there appears to be little, if any, direct use of the parent compound by consumers. Consumer exposure would probably be to the dyes or fluorescent whitening agents. Potential sources are clothing, especially when moistened by perspiration; packaging materials; some foods, such as fish; and insufficiently rinsed dishes. Daily human exposure to fluorescent whitening agents in Scotland was estimated at 4 μg/kg, but dermal and alimentary uptake is

probably very low (Kilbey, 1977). A review of the results of medical surveys and studies on the safety of the fluorescent whitening agents (especially those derived from diaminostilbene disulfonate) in Japan indicated very low potential toxicity to humans (Yamauchi and Shimizu, 1973). The increasing use of these agents, however, suggests that human exposures may increase as well (Kilbey, 1977).

Workers at a chemical plant where 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, was produced from *p*-nitrotoluene complained of sexual dysfunctions. Hammond *et al.* (1987) concluded that the workers were exposed to low, airborne concentrations of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt; 4,4′-dinitrostilbene-2,2′-disulfonic acid, disodium salt (DNS); and *p*-nitrotoluene sulfonic acid, sodium salt (PNTSA). Airborne concentrations were under 100 $\mu g/m^3$ in all cases; 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, concentrations were the lowest. These ionic salts have negligible vapor pressure, and therefore airborne exposure would only be in the form of aerosols. 4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, and DNS are related structurally to the synthetic hormone diethylstilbestrol (DES) (Hammond *et al.*, 1987), and in some studies 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, has been shown to have estrogenic activity (Smith and Quinn, 1992).

Metabolism and Chemical Disposition

Studies were conducted by NTP (unpublished, 1986) to determine the disposition of [^{14}C] 4,4′-diamino-2,2′-stilbenedisulfonic acid in rats after intravenous administration and after *ad libitum* administration in feed. Radiochemical purity of [^{14}C] 4,4′-diamino-2,2′-stilbenedisulfonic acid was 97.5% in the undiluted intravenous dosing solution and was 92.7% in the feed. Urinary excretion accounted for 96% to 97% of the dose at 24, 48, and 72 hours after intravenous dosing. Among organs evaluated in the intravenous study, alimentary tract tissue contained the highest concentration (14.7 ± 7.1 nCi/g) of chemical after 24 hours, and the kidneys contained the highest concentration of 4,4′-diamino-2,2′-stilbenedisulfonic acid (8.78 ± 0.97 nCi/g) after 72 hours. Seventy-two hours after the diet containing [^{14}C] 4,4′-diamino-2,2′-stilbenedisulfonic acid was removed, recovery of the radiolabel in the feces was 80% to 92%; recovery of the radiolabel in urine was minimal (less than 6%). Muscle tissue contained the greatest total amounts of radioactivity (0.55% to 0.77%) at 72 hours, while other tissues had only trace amounts.

Toxicity

The oral LD_{50} for the guinea pig is 47 g/kg. Other effects noted in guinea pigs were impaired liver function and depressed renal function (RTECS, 1990). In one study, two of six weanling female CD Sprague-Dawley rats administered intraperitoneal doses of 3,000 mg/kg 4,4′-diamino-2,2′-stilbenedisulfonic acid died within 24 hours; oral administration of 3,000 mg/kg was not toxic (Smith and Quinn, 1992).

Carcinogenicity

In an investigation of the anti-tumor activities of stilbene derivatives used as brighteners, 4,4′-bis-(4-anilino-6-*p*-sulfonanilino-1,3,5-triazinyl-2-amino)-stilbene 2,2′-disulfonate showed a marked tumor-inhibiting activity on solid forms of Ehrlich carcinoma, sarcoma 180, and carcinoma 63. Stilbene derivatives showed no effect on Ehrlich ascites carcinoma (Saito, 1970).

Genetic Toxicity

The two arylamine groupings in the structure of 4,4′-diamino-2,2′-stilbenedisulfonic acid classify the compound as one with potential for genotoxic activity (Ashby *et al.*, 1989). However, the mutagenicity test data for 4,4′-diamino-2,2′-stilbenedisulfonic acid, although limited, are all negative. The compound (maximum concentration 5 mg/plate) showed no mutagenic activity in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98, with or without S9 (Table E1; Zeiger *et al.*, 1987), and it did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells, with or without S9 (Tables E2 and E3; Loveday *et al.*, 1990). There are no genotoxicity data on metabolites of 4,4′-diamino-2,2′-stilbenedisulfonic acid.

Study Rationale

4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, was selected for study by the Consumer Product Safety Commission because of its high production volume and its use in the synthesis of dyes and bleaching agents widely used in the paper, leather, and textile industries. Also, no toxicologic or carcinogenesis information was available for 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, which is structurally similar to other azo dyes that demonstrate toxicologic or carcinogenic potential.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, was obtained from Ciba-Geigy Corporation in one lot (SW-81605), which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and are discussed in Appendix H. The study chemical, a yellow, microcrystalline powder, was identified as 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy.

The lot was approximately 76% pure, as determined by weight loss on drying, thin-layer chromatography, high-performance liquid chromatography, and elemental analysis. Weight loss on drying indicated the presence of approximately 14% water in the bulk chemical; elemental analysis indicated the presence of approximately 6% sodium chloride. Up to three organic impurities, constituting a total impurity level of approximately 4%, were observed by high-performance liquid chromatography. Using high-performance liquid chromatographic separation and direct inlet mass spectrometric analysis, one of the three organic impurities was tentatively identified as 4,4′-ethylene-dianiline-2-sulfonic acid. Stability studies performed by high-performance liquid chromatography indicated that 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, was stable as a bulk chemical for 2 weeks at temperatures up to 60° C when protected from light. Based on the stability study results, the bulk chemical was stored in the dark at room temperature at the testing laboratory throughout the study period. The stability of the bulk chemical was monitored periodically by high-performance liquid chromatography and ultraviolet spectroscopy during all phases of the studies. No change in the study material was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, with feed (Table H1). Homogeneity and stability studies were conducted by the analytical chemistry laboratory using ultraviolet spectroscopy and high-performance liquid chromatography, respectively. The homogeneity of the dose formulations was confirmed, and the stability of dose formulations stored for at least 14 days in the dark at temperatures to 5° C was established. The study laboratory conducted additional stability studies and confirmed the stability of the dose formulations for up to 3 weeks when stored at room temperature. During the 14-day and 13-week studies, the dose formulations were refrigerated in the dark for no longer than 2 weeks. During the 2-year studies, the dose formulations were initially stored in plastic bags protected from light at 1° C for no longer than 2 weeks; 8 months after study initiation the dose formulations were stored at 22° C.

The study laboratory conducted periodic analyses of the 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, dose formulations using ultraviolet spectroscopy as described in Appendix H. The dose formulations for the 14-day studies were analyzed once and all were within 10% of the target concentrations (Table H2). During the 13-week studies, the dose formulations were analyzed twice and all of the dose formulations for rats and mice were within 10% of the target concentrations (Table H3). During the 2-year studies, the dose formulations were analyzed at approximately 8-week intervals and 96% (27/28) of the dose formulations for rats and all of the dose formulations for mice were within 10% of the target concentrations (Table H4). Results of periodic referee analyses of the dose formulations performed by the analytical chemistry laboratory were in agreement with the results from the study laboratory (Table H5).

14-Day Studies

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Charles River Breeding Laboratories (Portage, MI) and observed for 15 days (rats) or 16 days (mice) before the studies began. Rats were 6 to 7 weeks old and mice were 7 to 8 weeks old at study initiation. Groups of five rats and five mice of each sex received feed containing 0, 6,250, 12,500, 25,000, 50,000, or 100,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, (Table 1). All groups received dosed feed for 14 days, followed by a 1-day observation period. Animals were housed five per cage; water and feed were available *ad libitum*. Clinical observations were conducted twice daily and recorded daily. Animals were weighed at the start of the study, on day 8, and day 16. Feed consumption was determined weekly. Complete necropsies were performed on all animals. The brain, heart, right kidney, liver, lungs, and thymus of survivors were weighed at necropsy. All animals in the 0 and 100,000 ppm dose groups received complete histopathologic examinations. Histopathology of the liver was performed on mice that received 6,250, 12,500, 25,000 or 50,000 ppm. Further details are presented in Table 1.

13-Week Studies

The 13-week studies were conducted to determine the cumulative toxic effects of repeated exposure to 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, and to determine appropriate chemical concentrations to be used in the 2-year studies. The experimental design of the 13-week studies is summarized in Table 1.

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Charles River Breeding Laboratories and were observed for 13 to 15 days before the studies began. Rats were 6 to 7 weeks old and mice were 7 to 8 weeks old at the beginning of the studies. Groups of 10 rats or 10 mice of each sex were given 0, 6,250, 12,500, 25,000, 50,000, or 100,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed for 13 weeks. Animals were housed five per cage; water and feed were available *ad libitum*. Animals were observed twice each day and clinical observations were recorded weekly. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K). Animals were weighed at the start of the study and weekly thereafter. Feed consumption was measured weekly. Further experimental details are presented in Table 1.

At the end of the 13-week studies, blood was collected from the orbital sinus plexus of all surviving animals for clinical pathology analyses. The animals were then killed and necropsies were performed on all study animals. The brain, heart, right kidney, liver, lungs, right testis, and thymus of survivors were weighed at necropsy. All animals that died or were killed prior to the end of the studies, all control animals, and all animals that received 100,000 ppm received complete histopathologic examinations. Liver sections from the 0, 25,000, and 100,000 ppm rat groups and all mice groups were stained with Oil Red O and periodic acid-Schiff (with and without diastase). The rectum/anus from all rat dose groups was examined microscopically. Tissues examined for mice in the 6,250, 12,500, 25,000, and 50,000 ppm dose groups were the anus, ovary, rectum, uterus, and liver (Oil Red O and periodic acid-Schiff stains were used on the liver). Additional information about histologic examination is provided in Table 1.

2-Year Studies

Study Design

Groups of 60 rats and 60 mice of each sex were administered 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed for 7 days a week for up to 103 weeks. Rats received doses of 0, 12,500, or 25,000 ppm and mice received doses of 0, 6,250, or 12,500 ppm. After 15 months of chemical administration, 10 rats and 10 mice of each sex were randomly selected from each group for interim evaluations.

Source and Specification of Animals

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Frederick Cancer Research Facility (Frederick, MD) for use in the 2-year studies. Rats were quarantined 16 days and mice were quarantined 13 days. Five rats and five mice per sex were randomly selected and killed for parasite evaluation and gross observation of disease.

Serology samples were collected for viral screens. Rats and mice were approximately 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program.

Animal Maintenance

Rats and mice were initially housed five per cage. Beginning in week 39, male mice were housed individually. Feed and water were available *ad libitum*. Cages were rotated weekly. Further details of animal maintenance are given in Table 1. Information on feed composition is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily and clinical findings were recorded weekly for 13 weeks, then monthly or as necessary. Animals were weighed at study initiation, weekly for 13 weeks, monthly through week 90, and every 2 weeks thereafter. Feed consumption was measured once a month (Appendix I).

Ten rats and 10 mice from each group were randomly selected and killed for interim evaluations after 15 months of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, administration. Blood was drawn from the orbital sinus plexus of rats and mice to determine the following hematology and clinical chemistry parameters: hemoglobin, hematocrit, erythrocyte count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, total leukocyte count, leukocyte differential count, blood urea nitrogen, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and sorbitol dehydrogenase. The brain, liver, and right kidney of each animal were weighed at necropsy. Further details of the interim evaluations are presented in Table 1.

Animals found in a moribund state, selected for the 15-month interim evaluations, or surviving to the end of the 2-year studies were killed. Necropsies were 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, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Histopathologic examinations were performed on all 15-month interim evaluation animals, all tissues with grossly visible lesions, all animals that died or were killed moribund prior to 21 months, and all control and high-dose animals killed at study termination. Tissues examined from all low-dose animals that died or were killed moribund after 21 months on study or were killed at the end of the studies are listed in Table 1.

Upon completion of the microscopic evaluation by the laboratory study pathologist, the pathology data were entered into the Toxicology Data Management System. The microscope slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet-tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slide and tissue counts were verified, and histotechnique was evaluated by the quality assessment laboratory. The adrenal gland and mammary gland of male and female rats, the pancreas and liver of male rats, the kidney of female rats, the lung of male and female mice, and the liver of male mice were evaluated microscopically by the quality assessment pathologist for both neoplastic and nonneoplastic lesions. All neoplastic diagnoses in all tissues in all rats and mice and all tissues from a randomly selected 10% of the control and high-dose rats and mice were reevaluated microscopically by a quality assessment pathologist.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnosis between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chair to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of

dose groups or previously rendered diagnoses. When the consensus opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are evaluated separately 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 of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses 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 that 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., skin or mammary tumors) prior to histologic sampling, or when lesions had multiple potential sites of occurrence (e.g., mononuclear cell leukemia), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence

The majority of tumors in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was a logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of tumor-bearing animals.

Tests of significance included pairwise 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 also were used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, see Haseman, 1984.

Historical Control Data

Although the concurrent control group is always 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. Consequently, control tumor incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for tumors appearing to show compound-related effects.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which have typically skewed distributions, were analyzed using the multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response (Dunnett's or Dunn's test).

Quality Assurance Methods

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

Genetic Toxicity

The genetic toxicity of 4,4′-diamino-2,2′-stilbenedisulfonic acid was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. The protocols for these studies and tabular presentations of the findings are in Appendix E.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

14-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
International Research and Development Corporation (Mattawan, MI)	International Research and Development Corporation (Mattawan, MI)	International Research and Development Corporation (Mattawan, MI)
Strain and Species		
Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source		
Charles River Breeding Laboratories (Portage, MI)	Charles River Breeding Laboratories (Portage, MI)	Frederick Cancer Research Facility (Frederick, MD)
Size of Study Groups		
5 males and 5 females	10 males and 10 females	60 males and 60 females
Doses		
0, 6,250, 12,500, 25,000, 50,000, or 100,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed	Same as 14-day studies	Rats: 0, 12,500, or 25,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed Mice: 0, 6,250, or 12,500 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed
Time Held Before Study		
Rats: 15 days Mice: 16 days	Rats: 13-14 days Mice: 15 days	Rats: 16 days Mice: 13 days
Average Age When Placed on Study		
Rats: 44-51 days Mice: 52-59 days	Rats: 42-49 days Mice: 50-58 days	Rats: 45 days Mice: 42 days
Date of First Dose		
Rats: 3 June 1981 Mice: 11 June 1981	Rats: 21-22 September 1981 Mice: 23 September 1981	Rats: 25 February 1983 Mice: 15 February 1983
Duration of Dosing		
14 days	13 weeks (7 days/week)	103 weeks (7 days/week)
Necropsy Dates		
Rats: 19 June 1981 Mice: 27 June 1981	Rats: 22-23 December 1981 Mice: 24 December 1981	15-month interim – Rats: 25 May 1984; Mice: 15 May 1984 2-year studies – Rats: 22-27 February 1985; Mice: 12-15 February 1985
Average Age at Necropsy		
Rats: 8-9 weeks Mice: 9-10 weeks	Rats: 19-20 weeks Mice: 20-21 weeks	Rats: 110-111 weeks Mice: 111 weeks

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Animals per Cage		
5	5	5 (male mice housed individually beginning 8 November 1983)
Method of Animal Distribution		
Animals assigned random numbers, grouped into blocks by body weight, and each block assigned to groups by random numbers.	Same as 14-day studies	Same as 14-day studies
Method of Animal Identification		
Rats: Ear tag Mice: Toe clip	Same as 14-day studies	Same as 14-day studies
Diet		
NIH-07 Rat and Mouse Ration, Open formula, mash (Zeigler Bros., Inc., Gardners, PA); available *ad libitum*	Same as 14-day studies	Same as 14-day studies
Water		
IRDC well water via automatic watering system (Edstrom Industries, Inc., Waterford, WI); available *ad libitum*	Same as 14-day studies	Village of Mattawan or IRDC well water via outside-the-cage automatic watering system (Edstrom Industries, Inc., Waterford, WI); available *ad libitum*
Cages		
Polycarbonate cages with Edstrom grommets (Hazelton System Inc., Aberdeen, MD)	Same as 14-day studies	Same as 14-day studies
Bedding		
BetaChips® hardwood laboratory bedding (Northeastern Products Corp., Warrensburg, NY); changed twice weekly	Same as 14-day studies	Same as 14-day studies
Cage Filters		
Reemay spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)	Same as 14-day studies	Same as 14-day studies
Animal Room Environment		
Rats: Average temperature - 75° F; Average humidity - 55.7% Mice: Average temperature -75.6° F; Average humidity - 57.9% Fluorescent light: 12 hours/day Room air changes: 6-12 changes/hour	Rats: Average temperature - 70.4° F; Average humidity - 43% Mice: Average temperature - 70.6° F; Average humidity - 43.1% Fluorescent light: 12 hours/day Room air changes: 6-12 changes/hour	Average temperature: 74° ± 2.5° F Average humidity: 50% ± 15.2% Fluorescent light: 12 hours/day Room air changes: 6-12 changes/hour

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Type and Frequency of Observation		
Observed twice/day; weighed initially and once/week; clinical observations recorded daily; feed consumption once/week by cage	Observed twice/day; weighed initially and once/week; clinical observations recorded once/week; feed consumption once/week by cage	Observed twice/day; weighed initially, once/week for 13 weeks, once/month through week 90, every 2 weeks thereafter; clinical observations recorded once/week for 13 weeks, once/month thereafter; feed consumption measured once/month
Necropsy		
Necropsy performed on all animals. The following organs were weighed: brain, heart, right kidney, liver, lungs, and thymus.	Necropsy performed on all animals. Organs weighed for all animals surviving to study termination: brain, heart, right kidney, liver, lungs, right testis, and thymus.	Necropsy performed on all animals. Organs weighed for all animals at 15-month interim evaluations: brain, right kidney, and liver.
Histopathology		
Complete histopathology performed on all control and 100,000 ppm animals. Tissues examined included: adrenal gland, bone and marrow (sternum), brain, clitoral or preputial gland (rats), colon, esophagus, gallbladder (mice), heart, jejunum, kidney, liver, lung, mammary gland, mandibular lymph node (rats), mesenteric lymph node, nasal cavity, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Livers were examined from mice in the 6,250, 12,500, 25,000 and 50,000 ppm dose groups.	Complete histopathology on all animals that died or were killed moribund during the study, all controls, and all 100,000 ppm rats and mice. Tissues examined included: adrenal gland, anus (mice), bone and marrow (sternum), brain, cecum, clitoral or preputial gland (rats), colon, duodenum, esophagus, gallbladder (mice), heart, ileum, jejunum, kidney, liver, lung, mammary gland, mesenteric lymph node, nasal cavity, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, rectum, salivary gland, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the anus/rectum was examined from rats in the 6,250, 12,500, 25,000, and 50,000 ppm groups; tissues examined from mice in the 6,250, 12,500, 25,000, and 50,000 ppm groups were anus, ovary, rectum, and uterus. Oil Red O and periodic acid-Schiff stains were used on liver tissues from rats in the control, 25,000, and 100,000 ppm groups and from all mice groups.	Complete histopathology performed on all animals killed at the 15-month interim evaluations, all animals that died or were killed moribund prior to 21 months on study, and control or high-dose animals killed at study termination. Tissues examined: adrenal gland, bone (costochondral junction), bone marrow (femur), brain, clitoral or preputial gland (rats), esophagus, gallbladder (mice), heart, kidney, large intestine, liver, lung, mammary gland, mesenteric lymph nodes, nasal cavity, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine, spleen, stomach, testis (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus. Tissues examined from low-dose rats that died or were killed moribund after 21 months or at study termination were gross lesions, adrenal gland, liver (males), kidney (females), mammary gland (females), pituitary gland (males), and spleen (males). Tissues examined from low-dose mice that died or were killed moribund after 21 months or at study termination were gross lesions, liver (males) and lung.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 4,4″-Diamino-2,2″-stilbenedisulfonic Acid, Disodium Salt (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Clinical Pathology		
None	Clinical pathology studies were conducted at 13 weeks on all rats and mice surviving to study termination. *Hematology:* None *Clinical chemistry:* glucose, total protein, albumin, albumin/globulin ratio, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase	Clinical pathology studies on rats and mice of each sex from each dose group were conducted at 15 months. *Hematology:* hematocrit, hemoglobin, erythrocyte count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, leukocyte count and differential *Clinical chemistry:* blood urea nitrogen, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and sorbitol dehydrogenase

RESULTS

RATS

14-Day Studies

All rats survived to the end of the studies. The mean body weight gains of males and females in the 100,000 ppm dose group and of males in the 50,000 ppm dose group were significantly lower than those of the controls (Table 2). The mean body weight gains of other dosed groups were similar to those of the controls. Feed consumption by the 50,000 and 100,000 ppm dose groups was lower than that of the controls during the first week; during the second week feed consumption by dosed and control males was similar and feed consumption by dosed females was higher than that of the controls.

Diarrhea was observed among rats that received 50,000 or 100,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt. Decreases in absolute or relative organ weights of males that received 100,000 ppm were considered secondary to the decrease in final mean body weight (Table F1). Absolute and relative liver weights were increased in females in the 100,000 ppm dose group. There were no gross or microscopic lesions related to chemical administration in dosed rats.

TABLE 2
Survival, Mean Body Weights, and Feed Consumption of Rats in the 14-Day Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	134 ± 4	211 ± 5	77 ± 3		22	18
6,250	5/5	137 ± 5	217 ± 6	81 ± 2	103	22	18
12,500	5/5	136 ± 5	211 ± 7	75 ± 3	100	21	18
25,000	5/5	138 ± 4	216 ± 4	79 ± 2	103	21	19
50,000	5/5	136 ± 3	199 ± 3	63 ± 2**	94	19	18
100,000	5/5	134 ± 4	185 ± 4**	51 ± 2**	88	15	18
Female							
0	5/5	112 ± 3	149 ± 3	37 ± 0		18	15
6,250	5/5	113 ± 4	149 ± 5	36 ± 2	100	19	18
12,500	5/5	111 ± 4	147 ± 5	36 ± 2	99	19	18
25,000	5/5	114 ± 3	153 ± 5	38 ± 3	103	20	17
50,000	5/5	111 ± 3	145 ± 2	34 ± 2	97	16	16
100,000	5/5	113 ± 3	145 ± 3	31 ± 2*	97	16	17

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Grams of feed per animal per day

13-Week Studies

One female in the 100,000 ppm dose group died in the fourth week of the study; there were no other deaths. The final mean body weights and mean body weight gains of male rats in the 50,000 and 100,000 ppm dose groups and females in the 100,000 ppm dose group were significantly lower than those of the controls (Table 3). Feed consumption by males that received 100,000 ppm was 35% lower than that of the controls during the first week, and feed consumption remained lower than controls through week 8 (Table 4). Feed consumption by females in the 100,000 ppm dose group was 27% lower than that of the controls during the first week. By week 4, feed consumption by females that received 100,000 ppm exceeded that of the controls. The lower final mean body weights of dosed rats may have resulted from a combination of decreased palatability and the replacement of a significant portion of the diet by nonnutritive chemical, with possible impairment of absorptive or digestive processes.

TABLE 3
Survival and Mean Body Weights of Rats in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	141 ± 5	381 ± 5	240 ± 3	
6,250	10/10	141 ± 5	382 ± 3	240 ± 5	100
12,500	10/10	138 ± 4	370 ± 4	232 ± 3	97
25,000	10/10	137 ± 4	377 ± 5	241 ± 3	99
50,000	10/10	140 ± 5	351 ± 7**	211 ± 4**	92
100,000	10/10	137 ± 5	289 ± 4**	152 ± 5**	76
Female					
0	10/10	111 ± 2	211 ± 3	101 ± 2	
6,250	10/10	110 ± 3	208 ± 4	97 ± 4	98
12,500	10/10	110 ± 3	211 ± 3	101 ± 2	100
25,000	10/10	110 ± 2	206 ± 3	96 ± 3	97
50,000	10/10	108 ± 4	203 ± 4	96 ± 3	96
100,000	9/10[c]	112 ± 3	197 ± 6*	85 ± 4**	94

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies.
[c] Week of death: 4

TABLE 4
Feed Consumption by Rats in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

Week of Study	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm	100,000 ppm
Male						
1	15.6	16.2	16.1	15.7	14.4	10.2
2	16.9	16.8	16.9	17.5	16.4	11.3
3	17.8	18.4	14.5	18.4	18.2	14.2
4	18.1	17.7	16.2	15.1	17.2	14.8
5	17.9	18.6	20.2	21.6	19.6	15.6
6	16.9	17.8	18.3	18.7	17.5	15.9
7	19.5	17.1	18.1	18.0	17.9	15.9
8	17.9	18.2	18.3	18.6	16.6	17.7
9	18.1	18.6	17.9	19.0	19.6	19.3
10	16.9	17.9	17.7	18.1	17.9	18.6
11	17.8	17.8	17.9	18.3	17.5	19.6
12	17.6	17.4	17.7	18.4	17.9	19.3
13	18.8	18.0	17.7	19.7	20.4	21.3
Mean ± SD	17.7 ± 1.0	17.7 ± 0.7	17.5 ± 1.4	18.2 ± 1.6	17.8 ± 1.6	16.4 ± 3.3
Female						
1	11.4	11.8	12.4	11.9	11.1	8.3
2	11.3	11.0	11.6	12.1	11.2	9.1
3	11.5	11.1	11.5	10.9	11.5	9.8
4	11.2	11.4	11.8	11.6	12.0	11.4
5	11.3	11.7	12.1	11.9	13.2	12.7
6	11.4	12.2	11.5	11.5	11.5	12.3
7	11.4	11.6	12.3	11.4	11.2	12.8
8	12.2	11.8	12.1	11.7	12.2	13.3
9	11.5	11.9	12.1	12.5	12.5	14.5
10	11.2	11.4	12.0	12.2	11.9	13.9
11	11.0	11.3	11.5	12.3	11.5	14.5
12	11.3	10.7	11.8	11.7	11.2	14.0
13	12.2	12.1	12.0	12.3	12.6	15.6
Mean ± SD	11.5 ± 0.4	11.5 ± 0.4	11.9 ± 0.3	11.8 ± 0.4	11.8 ± 0.7	12.5 ± 2.2

[a] Feed consumption given in grams per animal per day

Clinical findings in rats administered 100,000 ppm included redness around the anus and emaciation in males and females. Soft stools and diarrhea were noted in the 50,000 and 100,000 ppm dose groups.

Statistically significant changes in absolute or relative organ weights reflected the decreased final mean body weights and were not considered to be directly related to chemical administration (Table F2).

Statistically significant decreases in serum albumin and total protein levels occurred in most males and females receiving 100,000 ppm and may have been caused by the diarrhea, decreased feed consumption, or both. Other changes in clinical pathology values were not considered biologically significant (Table G1).

Chronic suppurative inflammation of the terminal portion of the rectum and anus occurred in most male and female rats that received 100,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt (Table 5). The lesions were characterized by infiltrates of neutrophils and macrophages in the mucosa and submucosa. In some of these animals, the regional lymph node was enlarged with dilated sinuses containing inflammatory cells. The rectal/anal inflammation may be related to the irritative effects of diarrhea, pruritus resulting from the diarrhea, or direct irritation of mucous membranes by unabsorbed chemical. The hypercellularity of the bone marrow represents an increase in the production of granulocytes and is a typical response to inflammation.

TABLE 5
Incidences of Selected Treatment-Related Lesions in Rats in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm	100,000 ppm
Male						
Rectum/anus						
Inflammation (chronic, suppurative)[a]	0/10	0/9	0/10	0/9	0/8	9/10**
Bone marrow, sternum						
Hypercellularity	0/10	–[b]	–	–	–	6/10**
Female						
Rectum/anus						
Inflammation (chronic, suppurative)	0/10	0/10	0/10	0/10	0/10	6/9**
Bone marrow, sternum						
Hypercellularity	0/10	–	–	–	–	9/9**

** Significantly different (P≤0.01) from the control group by the Fisher exact test
[a] Incidences given as number of animals with lesion/number of animals with tissues examined
[b] Not examined

Dose Selection Rationale: Dose levels of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, selected for rats in the 2-year studies were 12,500 and 25,000 ppm. In the 13-week study, the mean body weight gain relative to the controls was 37% lower in males receiving 100,000 ppm and 12% lower in males receiving 50,000 ppm. The decreased mean body weight gains, combined with the possible physiological and nutritional effects of the soft stool or diarrhea, precluded selecting higher doses. In female rats, mean body weight gain relative to the controls was 16% lower in the 100,000 ppm dose group and 5% lower in the 50,000 ppm dose group in the 13-week study. Lower body weight gains relative to the controls and the variable occurrence of diarrhea precluded the use of 50,000 ppm as a high dose in the 2-year study in females.

2-Year Studies

15-Month Interim Evaluation

There were no biologically significant changes in relative or absolute organ weights, clinical pathology parameters, or histopathology observations that were related to administration of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, at 15 months (Tables F3 and G2).

Body Weights, Feed Consumption, and Clinical Findings

Mean body weights of dosed groups were not markedly different from those of the controls throughout the studies (Tables 6 and 7 and Figure 1). However, the mean body weight of high-dose male rats was marginally lower than that of the controls (3%-7%) from week 4 to week 90. A similar marginal depression of mean body weight relative to controls was seen in high-dose females from week 26 to week 102. Feed consumption by males and females was generally within 5% of controls (Tables I1 and I2). No clinical findings were attributed to the administration of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt.

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Weeks on Study	0 ppm		12,500 ppm			25,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	143	60	141	99	60	141	99	60
2	175	60	175	100	60	175	100	60
3	209	60	207	99	60	205	98	60
4	239	60	238	100	60	231	97	60
5	260	60	257	99	60	251	96	60
6	280	60	275	98	60	274	98	60
7	290	60	283	98	60	279	96	60
8	307	60	302	98	60	299	97	60
9	319	60	317	99	60	313	98	60
10	330	60	326	99	60	321	98	60
11	345	59	336	98	60	330	96	60
12	355	59	352	99	60	337	95	60
13	358	59	351	98	60	345	96	60
14	366	59	360	98	60	349	95	60
18	382	59	377	99	60	364	95	60
22	381	59	379	100	59	356	93	60
26	409	59	407	99	59	393	96	60
30	426	59	423	99	59	403	95	60
34	434	59	428	99	59	412	95	60
38	440	59	439	100	59	424	97	60
42	450	59	450	100	59	432	96	60
46	460	59	459	100	59	441	96	60
50	463	59	460	99	58	447	97	60
54	472	59	471	100	58	458	97	60
58	486	58	475	98	58	459	95	60
62	478	58	475	99	58	459	96	60
66[a]	479	46	473	99	48	461	96	50
70	486	46	471	97	48	454	93	50
74	485	46	468	96	47	456	94	48
78	473	45	466	98	45	455	96	46
82	473	43	464	98	45	454	96	41
86	478	40	464	97	41	448	94	39
90	469	36	468	100	36	454	97	37
92	455	34	461	101	36	447	98	36
94	458	31	457	100	35	455	99	34
96	455	30	457	101	30	456	100	32
98	446	30	446	100	29	450	101	30
100	439	27	440	100	26	449	102	29
102	429	24	441	103	24	437	102	26
104	434	22	435	100	23	437	101	24
Terminal sacrifice		22			20			24
Mean for weeks								
1-13	278		274	99		269	97	
14-52	421		418	99		402	95	
53-104	464		461	99		452	97	

[a] Interim evaluation occurred during this week.

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Weeks on Study	0 ppm		12,500 ppm			25,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	111	60	111	99	60	111	100	60
2	128	60	129	100	60	129	101	60
3	143	60	143	100	60	144	101	60
4	157	60	158	101	60	155	99	60
5	165	60	164	100	60	163	99	60
6	175	60	171	98	60	168	96	60
7	179	60	177	99	60	173	97	60
8	187	60	185	99	60	180	96	60
9	188	60	190	101	60	186	99	60
10	194	60	194	100	60	191	99	60
11	200	60	199	99	60	194	97	60
12	204	60	203	100	60	200	98	60
13	205	60	204	100	60	202	99	60
14	209	60	207	99	60	203	98	60
18	210	59	213	102	59	208	99	60
22	219	59	217	100	59	215	98	60
26	227	59	226	99	59	221	97	60
30	235	59	232	99	59	226	96	60
34	239	59	234	98	59	230	96	60
38	247	59	245	99	59	239	97	60
42	256	59	248	97	59	243	95	60
46	261	59	257	98	59	251	96	60
50	269	59	262	97	59	259	96	60
54	286	59	276	97	59	272	95	59
58	297	59	286	96	59	280	94	59
62	306	59	294	96	59	291	95	59
66[a]	311	49	304	98	49	297	95	49
70	323	49	312	97	49	304	94	49
74	333	49	322	97	49	314	94	49
78	336	48	331	99	49	326	97	49
82	342	47	334	98	47	326	96	49
86	344	45	336	98	44	328	95	49
90	348	44	339	98	43	334	96	49
92	345	43	340	99	42	331	96	47
94	346	42	343	99	40	334	97	47
96	347	39	344	99	39	336	97	46
98	341	39	341	100	38	331	97	44
100	349	34	337	96	38	334	96	41
102	346	33	339	98	35	328	95	38
104	340	31	340	100	34	337	99	34
Terminal sacrifice		30			33			33
Mean for weeks								
1-13	172		171	99		169	98	
14-52	237		234	99		230	97	
53-104	332		325	98		318	96	

[a] Interim evaluation occurred during this week.

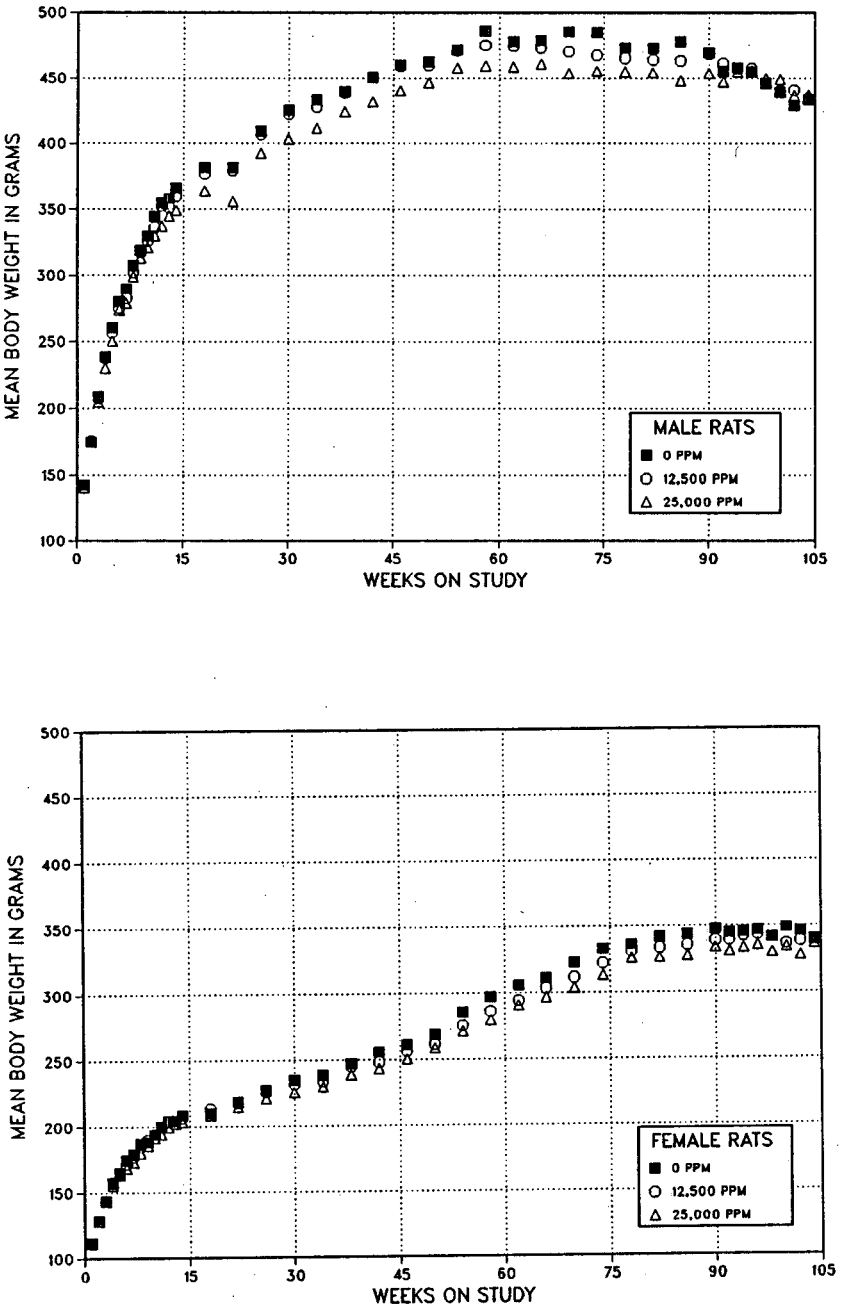


FIGURE 1
Growth Curves for Rats Administered 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt in Feed for 2 Years

Survival

The survival of dosed groups was similar to that of the controls, and the survival of all groups was 90% or greater through week 78 of the study (Table 8 and Figure 2).

Pathology and Statistical Analyses of Results

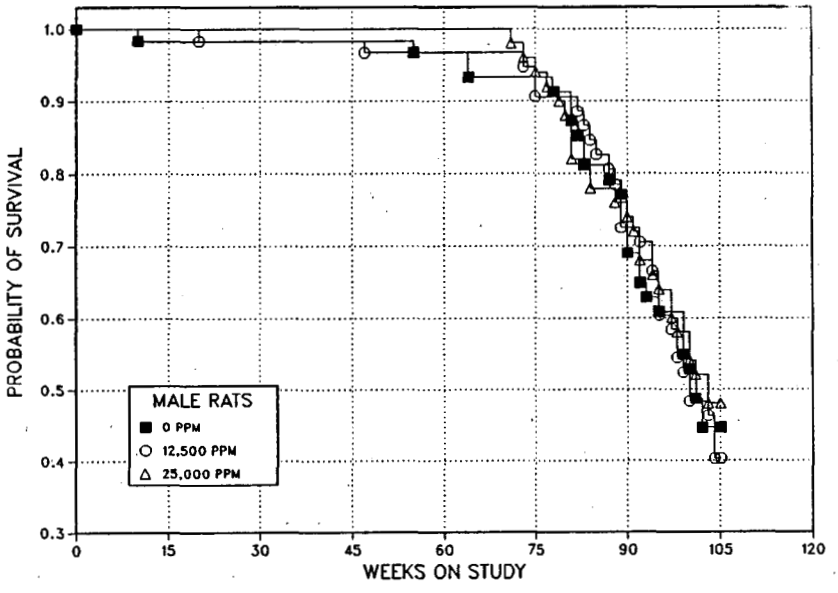
This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplastic or nonneoplastic lesions of the adrenal gland, mammary gland, stomach, and kidney in rats.

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

TABLE 8
Survival of Rats in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm	12,500 ppm	25,000 ppm
Male			
Animals initially in study	60	60	60
15-month interim evaluation[a]	10	10	10
Natural deaths	7	6	6
Moribund kills	21	24	20
Animals surviving to study termination	22	20	24[b]
Percent survival at end of study[c]	45	40	48
Mean survival (days)[d]	621	626	636
Survival analyses[e]	P=0.729N	P=0.945	P=0.785N
Female			
Animals initially in study	60	60	60
15-month interim evaluation[a]	10	10	10
Natural deaths	5	3	1
Moribund kills	15	14	16
Animals surviving to study termination	30	33[b]	33
Percent survival at end of study[c]	60	66	66
Mean survival (days)[d]	649	651	667
Survival analyses[e]	P=0.440N	P=0.701N	P=0.480N

[a] Censored from survival analyses
[b] Includes one animal that died during the last week of study
[c] Kaplan-Meier determinations. Survival rates adjusted for interim evaluations
[d] Mean of all deaths (uncensored, censored, terminal sacrifice)
[e] 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. A negative trend or lower mortality in a dose group is indicated by N.



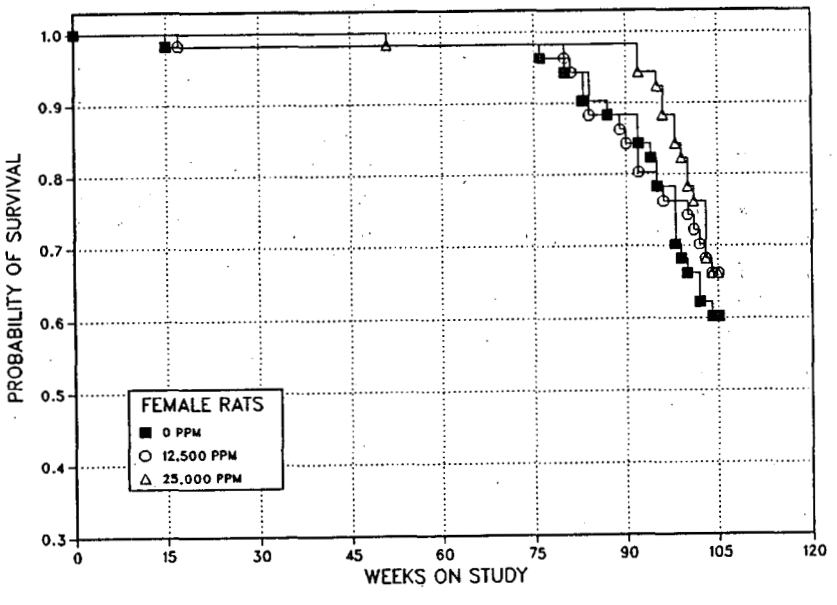


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt in Feed for 2 Years

Adrenal Gland: There was a marginal, statistically significant positive trend for malignant pheochromocytoma of the adrenal medulla in dosed males (Table 9). However, a positive trend was not seen for benign pheochromocytomas or for benign or malignant pheochromocytomas combined. Also, there was no corresponding dose-related increased incidence of adrenal medullary hyperplasia (17/48, 21/50, 13/50). Hyperplasia, benign pheochromocytoma, and malignant pheochromocytoma constitute a morphological and biological continuum, and there are no morphological criteria which clearly distinguish between these categories. Typically, cytological atypia, cellular pleomorphism, and heterogeneity of growth pattern increase as the size of the lesions increase. As an arbitrary standard, medullary neoplasms which extend through the adrenal capsule are diagnosed as malignant. In general, few malignant pheochromocytomas metastasize to distant organs in 2-year studies. In this study, one malignant pheochromocytoma in the high-dose males metastasized.

The marginally increased incidence of malignant pheochromocytomas in high-dose male rats was not considered to be related to the administration of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, because a) there was no concomitant increase in the incidence of adrenal medullary hyperplasia, benign pheochromocytomas, or benign or malignant pheochromocytomas combined; b) the incidences of malignant pheochromocytomas in low- and high-dose males are within the NTP historical control group range of 0% to 20% (Table A4); and c) there is no clear biological distinction between adrenal medullary neoplasms diagnosed as benign and those diagnosed as malignant with which to justify a conclusion based on malignant neoplasms alone.

Mammary Gland: The incidences of fibroadenomas in the low- and high-dose female rats were significantly increased relative to controls (11/50, 21/50, 21/50; Table B3). However, the incidence of fibroadenomas in the concurrent controls is well below the mean for NTP historical controls (39.3%), and the incidences in the dosed groups are only slightly above that of the overall historical controls and are well within the range of 8% to 58% (Table B4). Thus, the increased incidences of fibroadenomas were not considered to be chemical related.

Stomach: Ulcers of the forestomach or glandular stomach occurred in nine dosed male and five dosed female rats; a single control male had an ulcer of the glandular stomach (Table 10). Although the incidences in dosed groups are low and are not statistically significant compared with control groups, the spontaneous occurrence of erosions or ulcers of the stomach in control animals is uncommon. In addition, these ulcers were relatively large (1-5 mm in diameter) and were noted at gross observation. Thus, ulcers of the forestomach and glandular stomach may have been related to the administration of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt.

Kidney: Many dosed female rats and a few control females had intracellular crystals of undetermined origin in the pelvic epithelium which were diagnosed as mineralization (8/50, 30/50, 37/50). This lesion consisted of a minuscule amount of crystalline material within the cytoplasm of one or a few pelvic epithelial cells. The increased incidence is statistically significant and dose related, and thus was considered associated with chemical administration. However, this lesion was considered to be of little or no biological significance because a) quantitatively, the crystalline material was extremely minimal and b) an associated cellular degeneration or necrosis was not apparent.

TABLE 9
Adrenal Medulla Pheochromocytomas in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm	12,500 ppm	25,000 ppm
Hyperplasia			
Overall rates[a]	17/48 (35%)	21/50 (42%)	13/50 (26%)
Benign Pheochromocytoma			
Overall rates	16/48 (33%)	18/50 (36%)	19/50 (38%)
Adjusted rates[b]	50.5%	56.0%	59.6%
Terminal rates[c]	7/21 (33%)	7/20 (35%)	12/24 (50%)
First incidence (days)	540	583	520
Logistic regression tests[d]	P=0.396	P=0.491	P=0.438
Malignant Pheochromocytoma			
Overall rates	2/48 (4%)	4/50 (8%)	8/50 (16%)
Adjusted rates	8.9%	16.1%	27.6%
Terminal rates	1/21 (5%)	2/20 (10%)	4/24 (17%)
First incidence (days)	713	663	658
Logistic regression tests	P=0.037	P=0.358	P=0.059
Pheochromocytoma (Benign, Complex, or Malignant)[e]			
Overall rates	17/48 (35%)	21/50 (42%)	26/50 (52%)
Adjusted rates	52.7%	63.9%	73.6%
Terminal rates	7/21 (33%)	9/20 (45%)	15/24 (63%)
First incidence (days)	540	583	520
Logistic regression tests	P=0.067	P=0.334	P=0.084

[a] Number of lesion-bearing animals/number of animals examined at site
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard tumors in animals dying prior to terminal kill as nonfatal.
[e] Historical incidence from 2-year NTP feed studies of untreated control groups (mean ± standard deviation): 306/788 (38.8% ± 8.4%), range 22%–48%

TABLE 10
Stomach Ulcers in Rats in the 2-Year Feed Studies
of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm	12,500 ppm	25,000 ppm
Male			
Forestomach: Ulcer			
Overall rates[a]	0/50 (0%)	4/50 (8%)	1/50 (2%)
Glandular Stomach: Ulcer			
Overall rates	1/50 (2%)	1/50 (2%)	4/50 (8%)
Forestomach or Glandular Stomach: Ulcer			
Overall rates	1/50 (2%)	5/50 (10%)	4/50 (8%)
Female			
Forestomach: Ulcer			
Overall rates	0/50 (0%)*	1/50 (2%)	4/50 (8%)
Glandular Stomach: Ulcer			
Overall rates	0/50 (0%)	0/50 (0%)	2/50 (4%)
Forestomach or Glandular Stomach: Ulcer			
Overall rates	0/50 (0%)*	1/50 (2%)	4/50 (8%)

* Trend was significant ($P \leq 0.05$) by logistic regression; no pairwise differences were significant.
[a] Number of lesion-bearing animals/number of animals necropsied

MICE

14-Day Studies

All mice survived to the end of the studies. The mean body weight gains of males and females that received 100,000 ppm were significantly lower than those of the controls (Table 11). The values for feed consumption by males and females in the 100,000 ppm dose groups were higher than those of the controls throughout the studies. These values, however, represent both feed consumed and feed spilled. The apparent increase in these values is likely due to reduced palatability and excess feed loss.

Clinical findings in females that received 50,000 or 100,000 ppm and in males that received 100,000 ppm included soft stools. Diarrhea and swelling or hyperemia of the perineum were observed less frequently in mice that received 100,000 ppm. Decreases in absolute and relative organ weights were considered secondary to the decreased mean body weights (Table F4).

TABLE 11
Survival, Mean Body Weights, and Feed Consumption of Mice in the 14-Day Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	21.0 ± 0.6	24.2 ± 0.7	3.2 ± 0.9		3.0	2.6
6,250	5/5	21.2 ± 0.6	24.2 ± 0.7	3.0 ± 0.5	100	2.6	2.4
12,500	5/5	20.6 ± 0.6	24.4 ± 0.5	3.8 ± 0.4	101	2.9	2.8
25,000	5/5	21.0 ± 0.3	23.4 ± 0.2	2.4 ± 0.2	97	2.6	2.3
50,000	5/5	20.8 ± 0.7	23.8 ± 0.7	3.0 ± 0.3	98	3.0	2.7
100,000	5/5	20.8 ± 0.6	22.2 ± 0.9*	1.4 ± 0.4*	92	4.7	3.4
Female							
0	5/5	18.4 ± 0.7	21.0 ± 0.6	2.6 ± 0.5		2.3	2.7
6,250	5/5	18.0 ± 0.3	20.2 ± 0.6	2.2 ± 0.4	96	3.1	3.2
12,500	5/5	18.2 ± 0.7	20.0 ± 0.8	1.8 ± 0.4	95	3.8	2.3
25,000	5/5	18.4 ± 0.7	20.8 ± 0.6	2.4 ± 0.9	99	3.0	2.6
50,000	5/5	18.4 ± 0.8	20.0 ± 0.7	1.6 ± 0.2	95	2.8	3.2
100,000	5/5	18.4 ± 0.7	19.4 ± 0.6	1.0 ± 0.3*	92	3.5	3.5

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Grams of feed per animal per day

13-Week Studies

Six males in the 100,000 ppm dose group died; three of these deaths occurred during week 4 and the remainder occurred during weeks 6, 8, and 11 (Table 12). One female receiving 100,000 ppm died during week 10. This mortality suggests cumulative effects, as there were no deaths in the 14-day studies. For the 13-week period, there was an actual mean body weight loss in males and females in the 100,000 ppm dose groups. Mean body weight gains of males and females that received 50,000 ppm were 31% less than those of the respective controls, and in the 25,000 ppm dose groups, the weight gains were 18% lower in males and 8% lower in females than those in the respective control groups. Values for feed consumption (plus spillage) by the 100,000 ppm dose groups exceeded the control values by 250% for males and 150% for females at 13 weeks (Table 13). Increases in feed consumption and spillage relative to the controls were less marked in the lower dose groups. These increases were attributed to reduced palatability and excess feed loss rather than to increased feed consumption.

Significant clinical findings in the 100,000 ppm dose group included body tremors (probably caused by weakness, rather than neurological impairments), lethargy, emaciation, and diarrhea. Statistically significant changes in absolute or relative organ weights were considered to reflect the decreased final mean body weights (Table F5). Statistically significant decreases in serum albumin and total protein levels in the 100,000 ppm groups (Table G3) were likely due to diarrhea, reduced feed intake, emaciation, or a combination of these factors. Other changes in clinical pathology values were not considered biologically significant.

TABLE 12
Survival and Mean Body Weights of Mice in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)
Male					
0	10/10	20.8 ± 0.5	30.1 ± 0.6	9.3 ± 0.3	
6,250	10/10	20.9 ± 0.5	29.0 ± 0.8	8.1 ± 0.4	96
12,500	10/10	20.5 ± 0.4	29.0 ± 0.7	8.5 ± 0.7	96
25,000	10/10	20.5 ± 0.5	28.1 ± 0.8	7.6 ± 0.5°	93
50,000	10/10	20.3 ± 0.6	26.7 ± 0.7°°	6.4 ± 0.5°°	89
100,000	4/10[c]	20.5 ± 0.5	20.0 ± 0.8°°	-0.5 ± 0.3°°	66
Female					
0	10/10	18.0 ± 0.3	24.0 ± 0.4	6.0 ± 0.3	
6,250	10/10	18.0 ± 0.5	23.3 ± 0.6	5.3 ± 0.2	97
12,500	10/10	17.4 ± 0.4	23.3 ± 0.4	5.9 ± 0.4	97
25,000	10/10	17.9 ± 0.3	23.4 ± 0.6	5.5 ± 0.4	97
50,000	10/10	17.3 ± 0.4	21.4 ± 0.4°°	4.1 ± 0.2°°	89
100,000	9/10[d]	17.7 ± 0.2	16.6 ± 0.6°°	-1.2 ± 0.5°°	69

° Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
°° P≤0.01
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies.
[c] Week of death: 4, 4, 4, 6, 8, 11
[d] Week of death: 10

TABLE 13
Feed Consumption by Mice in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

Week of Study	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm	100,000 ppm
Male						
1	3.2	3.0	3.2	3.0	2.9	3.2
2	3.3	3.6	3.6	4.2	4.0	5.5
3	3.4	3.3	3.3	3.9	3.9	5.1
4	3.2	3.2	3.4	3.8	2.7	3.6
5	3.9	3.7	4.1	4.6	4.9	4.5
6	3.6	3.6	3.8	4.2	4.3	5.8
7	3.9	3.8	4.3	4.8	4.2	7.2
8	3.6	3.7	4.2	4.6	4.2	7.9
9	4.0	3.7	4.2	4.1	4.3	6.9
10	3.7	3.9	4.2	4.5	4.6	8.5
11	4.1	3.8	4.3	4.8	4.7	10.1
12	3.9	3.7	4.0	4.6	4.0	9.3
13	4.2	4.1	4.3	5.1	5.0	10.6
Mean ± SD	3.7 ± 0.3	3.6 ± 0.3	3.9 ± 0.4	4.3 ± 0.5	4.1 ± 0.7	6.8 ± 2.4
Female						
1	3.1	2.5	2.9	2.8	3.2	2.8
2	3.2	3.0	3.1	3.4	4.7	–[b]
3	3.1	2.9	3.1	3.1	4.3	4.6
4	2.8	2.6	2.6	2.9	3.4	3.1
5	3.4	3.1	3.2	3.6	4.5	4.5
6	3.1	3.0	2.9	3.3	4.4	3.9
7	3.3	3.3	3.3	3.7	3.7	4.4
8	3.3	3.3	3.1	3.6	4.2	4.2
9	3.3	2.9	3.2	3.3	3.9	4.0
10	3.2	3.2	3.3	3.9	5.4	4.8
11	3.7	3.6	3.4	3.6	3.9	5.0
12	3.4	3.3	3.2	3.5	3.9	4.3
13	3.6	3.1	3.6	4.0	4.7	5.4
Mean ± SD	3.3 ± 0.2	3.1 ± 0.3	3.1 ± 0.3	3.4 ± 0.4	4.2 ± 0.6	4.3 ± 0.7

[a] Feed consumption given in grams per animal per day, based on average consumption data per week
[b] No data available

Chronic inflammation with occasional ulceration was observed in the rectal or anal mucosa of mice receiving 25,000 ppm or more 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt (Table 14). As with the rats, this may be related to the irritative effects of diarrhea, direct irritation of mucous membranes by unabsorbed chemical, or scratching due to pruritus.

Atrophy of the ovaries and uterus occurred in the 100,000 ppm dose group, and some females that received 6,250 to 50,000 ppm showed cystic endometrial hyperplasia (Table 14). Uterine and ovarian atrophy were considered to be related to body weight loss and inanition. The cystic endometrial hyperplasia was mild in all cases, and while the etiology is undetermined, the hyperplasia was

TABLE 14
Incidences of Selected Treatment-Related Lesions in Mice in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm	100,000 ppm
Male						
Rectum						
Inflammation, ulcerative[a]	0/10	0/9	0/10	2/10	0/10	4/9*
Anus						
Inflammation, ulcerative	0/8	1/8	0/10	2/6	4/6*	9/9**
Thymus						
Atrophy	0/10	–[b]	–	–	–	5/8**
Female						
Rectum						
Inflammation, ulcerative	0/10	0/10	0/9	2/10	0/10	3/10
Anus						
Inflammation, ulcerative	0/9	0/9	0/10	3/10	5/10*	9/9**
Ovary						
Atrophy	0/10	0/10	1/10	0/10	1/8	10/10**
Uterus						
Cystic endometrial hyperplasia	0/10	1/10	1/10	5/10*	2/10	0/10
Endometrial atrophy	0/10	0/10	0/10	0/10	0/10	10/10**

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Incidences given as number animals with lesion/number of animals with tissues examined
[b] Not examined

possibly the result of estrogenic effects of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt. A positive dose-related response may have been obscured by the decreased final mean body weights in the 50,000 and 100,000 ppm dose groups.

Dose Selection Rationale: The observation of deaths, decreased mean body weight gain relative to that of the controls, and ulcerative anal and rectal mucosal inflammatory lesions precluded the selection of doses of 25,000 ppm or above. Therefore, doses of 0, 6,250, and 12,500 ppm were selected for the 2-year studies in mice.

2-Year Studies

15-Month Interim Evaluation

There were no biologically significant changes in absolute or relative organ weights, clinical pathology parameters, or histology observations that were related to the administration of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, at 15 months (Tables F6 and G4).

Body Weights, Feed Consumption, and Clinical Findings

Mean body weights of all dosed groups were generally similar to those of the controls throughout the study (Tables 15 and 16 and Figure 3). However, the mean body weight of female mice receiving 12,500 ppm was consistently 4% to 5% lower than that of the controls after week 22. Feed consumption was similar between control and dosed groups (Tables I3 and I4). No clinical findings were attributed to the administration of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt.

TABLE 15
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Weeks on Study	0 ppm		6,250 ppm			12,500 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	21.3	60	20.9	98	60	21.2	100	60
2	22.7	60	22.7	100	60	22.8	100	60
3	24.1	60	24.3	101	60	24.2	100	60
4	25.4	60	25.2	99	60	24.8	98	58
5	26.5	60	26.3	99	60	26.4	100	58
6	27.4	60	27.1	99	60	27.0	99	58
7	28.0	60	28.0	100	60	27.0	96	58
8	29.1	59	29.0	100	60	28.6	98	58
9	29.5	58	29.3	99	60	29.4	100	58
10	30.0	58	29.4	98	60	29.3	98	58
11	30.1	57	30.2	100	60	29.8	99	57
12	30.9	57	30.5	99	60	30.3	98	57
13	31.0	57	30.8	99	60	30.8	99	57
14	31.5	57	30.8	98	60	31.4	100	57
18	32.0	57	32.3	101	60	32.5	102	57
22	33.0	56	32.3	98	60	31.9	97	55
26	33.3	56	33.0	99	60	32.5	98	55
30	33.7	56	33.4	99	59	33.3	99	55
34	34.6	56	34.7	100	58	34.2	99	55
38	35.7	56	34.9	98	58	34.7	97	55
42	35.7	56	35.1	98	57	35.2	99	55
46	35.9	56	35.5	99	57	35.2	98	55
50	37.4	54	36.3	97	57	36.1	97	55
54	40.1	54	38.8	97	57	38.0	95	55
58	37.2	54	37.9	102	57	37.7	101	55
62	40.3	54	40.3	100	57	38.3	95	55
66[a]	40.3	44	39.8	99	46	39.5	98	44
70	41.8	44	41.5	99	46	41.6	100	44
74	42.8	44	42.1	98	46	41.8	98	44
78	42.6	44	42.3	99	46	42.4	100	44
82	42.2	44	41.7	99	46	41.4	98	44
86	41.7	43	40.9	98	46	40.7	98	43
90	41.0	43	40.3	98	44	39.5	96	43
92	40.7	43	39.8	98	43	39.4	97	43
94	39.5	43	39.7	101	43	38.7	98	42
96	39.9	43	39.5	99	43	38.8	97	42
98	39.8	43	39.3	99	43	38.7	97	42
100	39.8	43	39.3	99	43	38.6	97	42
102	38.4	43	37.8	98	42	37.3	97	42
104	38.3	43	37.8	99	40	37.1	97	42
Terminal sacrifice		43			40			42
Mean for weeks								
1-13	27.4		27.2	99		27.0	99	
14-52	34.3		33.8	99		33.7	98	
53-104	40.4		39.9	99		39.4	98	

[a] Interim evaluation occurred during this week.

TABLE 16
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Weeks on Study	0 ppm		6,250 ppm			12,500 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	16.7	60	17.1	102	60	17.3	104	60
2	17.8	60	17.9	101	60	17.6	99	60
3	19.0	60	18.7	98	60	18.9	100	60
4	19.3	60	19.3	100	60	19.4	101	60
5	20.3	60	20.3	100	60	20.2	100	60
6	21.0	60	20.5	98	60	20.6	98	60
7	21.6	60	21.0	97	60	21.3	99	60
8	22.3	60	22.0	99	60	22.2	100	60
9	22.8	60	22.9	100	60	22.7	100	60
10	22.8	60	22.9	100	60	23.0	101	60
11	23.1	60	23.3	101	60	23.1	100	60
12	23.7	60	23.7	100	60	23.4	99	60
13	23.9	60	23.9	100	60	23.3	98	60
14	24.3	60	24.1	99	60	24.3	100	60
18	25.6	60	26.3	103	60	25.1	98	60
22	26.7	60	26.7	100	60	25.4	95	60
26	27.2	60	27.3	100	59	25.9	95	60
30	27.5	60	27.6	100	59	26.3	96	60
34	29.3	60	29.9	102	59	28.1	96	60
38	30.9	60	31.4	102	59	29.0	94	60
42	31.5	60	32.0	102	59	30.1	96	60
46	29.8	60	31.7	106	59	30.4	102	60
50	32.1	60	32.6	102	59	31.2	97	60
54	34.5	60	34.9	101	59	32.6	95	60
58	33.9	60	34.2	101	59	32.8	97	59
62	35.4	59	34.7	98	59	32.7	92	59
66[a]	35.4	49	34.9	99	48	33.2	94	49
70	37.3	49	37.7	101	48	34.4	92	49
74	37.8	49	37.6	100	48	35.7	94	49
78	37.7	49	38.0	101	48	35.5	94	48
82	37.3	49	38.8	104	47	36.0	97	48
86	37.5	48	38.3	102	47	35.9	96	48
90	37.9	46	38.0	100	46	35.7	94	47
92	38.0	46	38.1	100	46	36.1	95	47
94	37.8	46	38.0	101	45	35.4	94	45
96	37.9	46	38.1	101	44	35.7	94	44
98	37.5	46	37.8	101	43	35.7	95	41
100	37.1	46	38.0	102	43	36.0	97	40
102	37.0	45	36.8	100	43	34.8	94	40
104	37.5	43	37.3	100	43	35.6	95	38
Terminal sacrifice		43			43			38
Mean for weeks								
1-13	21.1		21.0	100		21.0	100	
14-52	28.5		29.0	102		27.6	97	
53-104	36.9		37.1	101		34.9	95	

[a] Interim evaluation occurred during this week.

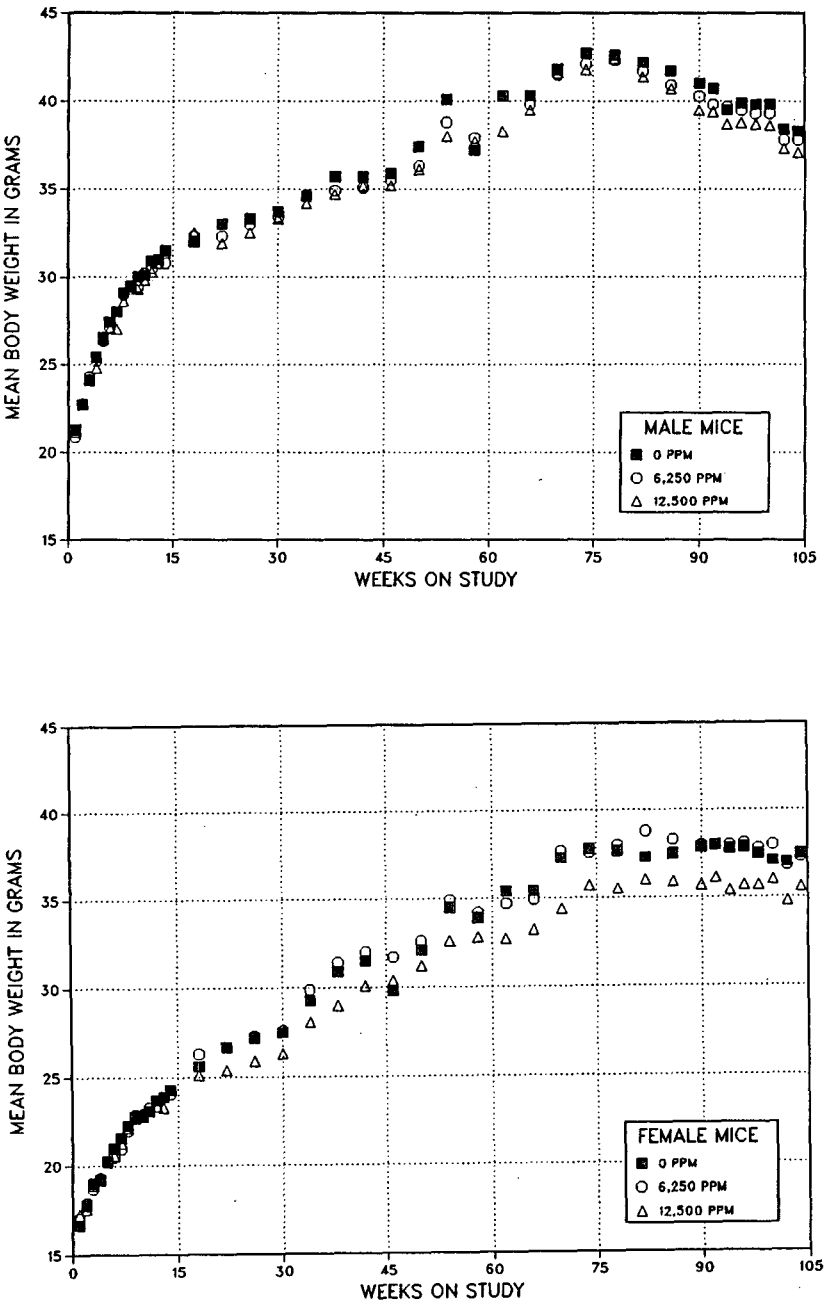


FIGURE 3
Growth Curves for Mice Administered 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt in Feed for 2 Years

Survival

Survival was similar between control and dosed groups of male and female mice (Table 17 and Figure 4).

Pathology and Statistical Analyses of Results

This section describes the biologically noteworthy changes in the incidences of neoplastic or nonneoplastic lesions of the lung in mice.

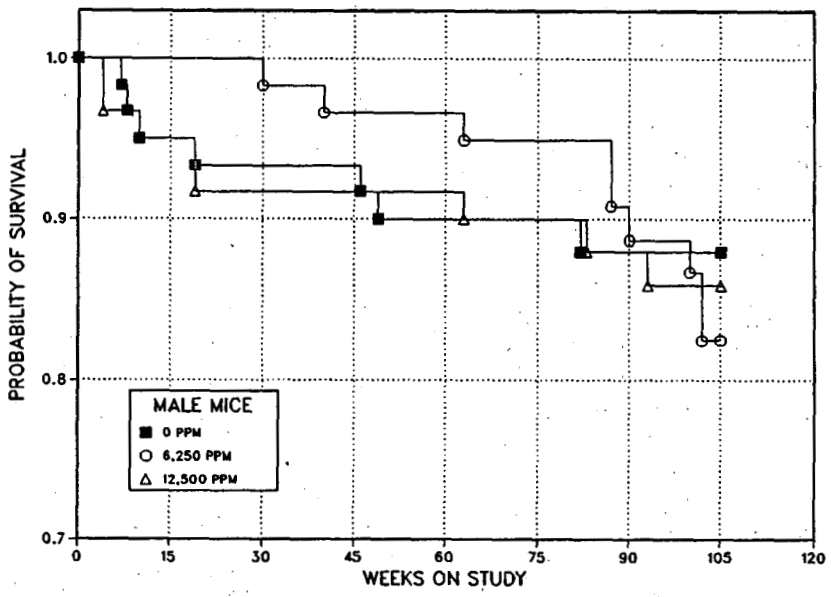
Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group are presented in Appendixes C for male mice and D for female mice.

Hypertrophy of the wall of the small arteries and arterioles of the lung was present in female mice in the 2-year study. The highest incidence of this

TABLE 17
Survival of Mice in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm	6,250 ppm	12,500 ppm
Male			
Animals initially in study	60	60	60
15-month interim evaluation[a]	10	10	10
Natural deaths	4	6	2
Moribund kills	3	3	6
Missing[a]	0	1	0
Animals surviving to study termination	43	40	42
Percent survival at end of study[b]	88	83	86
Mean survival (days)[c]	624	647	620
Survival analyses[d]	P=0.888	P=0.842	P=0.992
Female			
Animals initially in study	60	60	60
15-month interim evaluation[a]	10	10	10
Natural deaths	5	5	6
Moribund kills	2	2	5
Missing[a]	0	0	1
Animals surviving to study termination	43	43	38
Percent survival at end of study[b]	86	87	78
Mean survival (days)[c]	671	661	664
Survival analyses[d]	P=0.348	P=0.827	P=0.417

[a] Censored from survival analyses
[b] Kaplan-Meier determinations. Survival rates adjusted for missing animals and interim evaluations.
[c] Mean of all deaths (uncensored, censored, terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns.



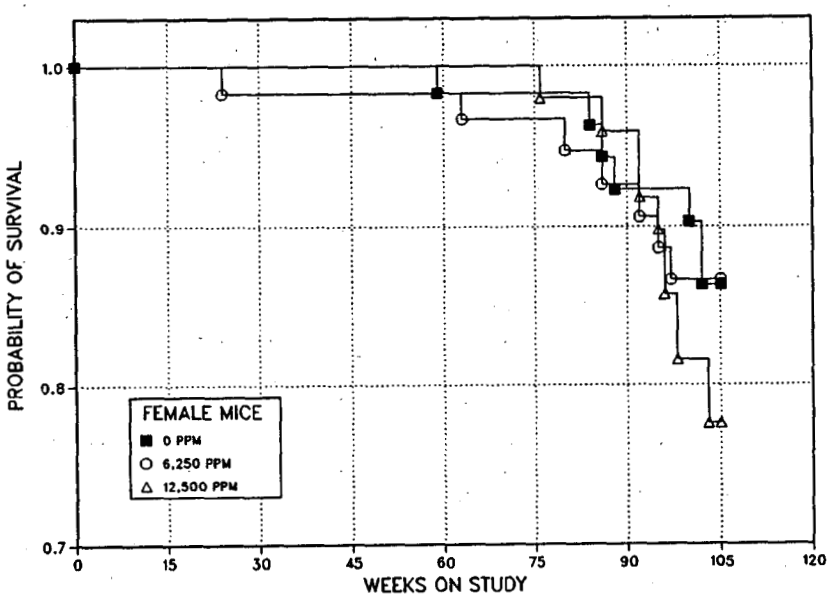


FIGURE 4
Kaplan-Meier Survival Curves for Mice Administered 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt in Feed for 2 Years

lesion occurred in females that received 12,500 ppm, but hypertrophy also occurred in control females (0 ppm, 10/50; 6,250 ppm, 8/49; 12,500 ppm, 21/49; Table D4).

Affected vessels were distributed randomly and had minimal to mild circumferential thickening of mural smooth muscle; it was not determined if this thickening was due to myocyte hypertrophy, hyperplasia, or both. While there are no historical data for this lesion, it appears to occur spontaneously. The response was not dose related and the incidence in the control group was relatively high; thus the biological significance of the marginal increase in the 12,500 ppm dose group was not determined.

Incidences of alveolar/bronchiolar adenomas were decreased in dosed mice (males: 12/50, 3/48, 7/50; females: 12/50, 3/49, 5/49; Tables C3 and D3). These decreased incidences are not dose-related and the incidences in the control groups are at the high end of the historical control range of 4% to 24% for males and 0% to 24% for females. Therefore, the decreased incidences were not considered related to administration of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt.

Genetic Toxicology

4,4′-Diamino-2,2′-stilbenedisulfonic acid was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 when tested in a preincubation protocol at concentrations of 100 to 5,000 μg/plate in the presence and the absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table E1; Zeiger *et al.*, 1987). 4,4′-Diamino-2,2′-stilbenedisulfonic acid was tested for induction of sister chromatid exchanges (Table E2) and chromosomal aberrations (Table E3) in Chinese hamster ovary cells in two laboratories; results in both laboratories were negative for each endpoint. In the first laboratory, 4,4′-diamino-2,2′-stilbenedisulfonic acid was tested for induction of sister chromatid exchanges and chromosomal aberrations using standard harvest times, with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9, at concentrations up to 1,020 μg/mL (Loveday *et al.*, 1990). In the second laboratory, higher doses, up to 5,000 μg/mL 4,4′-diamino-2,2′-stilbenedisulfonic acid were tested with and without S9; a delayed harvest protocol was used to obtain sufficient cells for analysis at the highest dose in the sister chromatid exchanges trials and the chromosomal aberrations trial conducted in the absence of S9.

DISCUSSION AND CONCLUSIONS

4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, is used primarily in the synthesis of dyes, such as Chrysophenine (Direct Yellow 12) or the nonethylated dye Brilliant Yellow (Direct Yellow 4), and in the synthesis of optical brighteners or fluorescent whitening agents (FWAs). FWAs are added to paper, leather, fabrics, plastics, and laundry detergents to enhance colors and whiteness (*Kirk-Othmer*, 1983a,b). 4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, was nominated by the Consumer Product Safety Commission for study because of the high production volume, its use in the synthesis of dyes and bleaching agents, the lack of toxicologic and carcinogenesis data, and its structural similarity to other azo dyes with toxicologic or carcinogenic potential.

During the 14-day rat and mouse studies, toxic effects were generally mild and included diarrhea and reduced body weight gains in animals administered 100,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed. These effects were more pronounced in the 13-week studies. Some mice receiving 100,000 ppm died, and the mean body weight gains of males and females receiving 50,000 or 100,000 ppm as well as males receiving 25,000 ppm were significantly lower than those of the controls. Poor palatability of feed was considered to have contributed to the decreased body weight gain. Most of the measured increase in feed consumption was probably due to wasting by animals searching for unadulterated feed. Inflammatory lesions of the rectum/anus occurred in rats receiving 100,000 ppm and in mice receiving 25,000 ppm or more; these lesions were frequently ulcerative in mice. It was not determined to what extent these lesions may have been caused by a direct caustic or irritative effect of unabsorbed 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, or by irritation associated with diarrhea or soft stools. The inflammatory lesions, decreased body weight gains, and increased feed consumption were limiting factors in dose selection for the 2-year studies.

In the 2-year studies, the high dose selected was 25,000 ppm for rats and 12,500 ppm for mice; survival was not decreased and toxic effects in rats were limited to slightly lower body weights and a marginal increased incidence of gastric ulceration. There were no significant nonneoplastic effects in mice and no neoplastic effects in rats or mice in the 2-year studies. Although the animals may have tolerated slightly higher doses, results of the 13-week studies indicate that the doses could not have been doubled.

Information on the toxicity of 4,4′-diamino-2,2′-stilbenedisulfonic acid is scant; however, available information suggests the chemical is only mildly toxic in humans (Yamauchi and Shimizu, 1973) and other animals (Kilbey, 1977). This is consistent with the findings in these NTP toxicity and carcinogenicity studies. Kilbey indicated that dermal and alimentary uptake is probably very low, and NTP studies (1986) conducted to determine the disposition of [^{14}C] 4,4′-diamino-2,2′-stilbenedisulfonic acid in rats after *ad libitum* administration in the feed revealed very low absorption rates. Seventy-two hours after the diet containing [^{14}C] 4,4′-diamino-2,2′-stilbenedisulfonic acid was removed, recovery of radiolabel in the feces was 80% to 92%; less than 6% of the radiolabel was recovered in the urine. Muscle tissue contained 0.55% to 0.77% radioactivity at 72 hours, while other tissues had only trace amounts. An earlier (1983) NTP disposition study revealed negligible absorption via the alimentary route. Thus, low systemic availability of 4,4′-diamino-2,2′-stilbenedisulfonic acid via the common exposure routes may minimize its potential toxic effects.

In a report of sexual dysfunction among male chemical workers manufacturing the stilbene derivative, 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, clinical symptoms included impotence and decreased libido (Hammond *et al.*, 1987). In this report, less than 350 ng/dL serum testosterone (normal range 350-1,050 ng/dL) was reported in

37% of all exposed men; however, there was no significant difference between the testosterone levels of men who reported impotence and of those who did not. These effects are similar to those that might occur with estrogenic compounds such as diethylstilbestrol (DES); however, other effects of DES, such as gynecomastia (Zaebst *et al.*, 1980), did not occur in workers exposed to 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt. Airborne concentrations of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, were low; however, a fine yellowish film of the chemical on surfaces throughout the work area made dermal or oral exposure possible (Quinn *et al.*, 1990).

4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, and 4,4′-dinitrostilbene-2,2′-disulfonic acid, disodium salt (DNS), are structurally related to the synthetic hormone DES. Animal assays were used to determine the estrogenic activity, shown by increased uterine weight in sexually immature female rats, of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, and three of its synthetic precursors: DNS, 2-methyl-5-nitrobenzenesulfonic acid (MNBSA), and 4-nitrotoluene (Smith and Quinn, 1992). Estrogens have been implicated as a possible etiologic factor in the development of endometrial carcinoma, breast carcinoma, ovarian cancer, vaginal adenosis, and vaginal clear-cell carcinoma in humans. In the animal assays, 4,4′-diamino-2,2′-stilbenedisulfonic acid and 4-nitrotoluene exhibited uterotrophic effects, but both were much weaker than DES. 4-Nitrotoluene is not structurally similar to any estrogenic chemical; however, chemicals with quite diverse structures have been found to possess estrogenic activity (Mueller and Kim, 1978; Katzenellenbogan, 1980). Estrogenic activity of 4,4′-diamino-2,2′-stilbenedisulfonic acid and 4-nitrotoluene has been substantiated in preliminary *in vitro* experiments which showed that they bind, albeit weakly, to estrogen receptors isolated from rabbit uteri. The responses to oral doses of 4,4′-diamino-2,2′-stilbenedisulfonic acid were not appreciably different from the responses to the same doses given intraperitoneally. Also, a sample of 4,4′-diamino-2,2′-stilbenedisulfonic acid taken from the above-mentioned manufacturing plant was found to have uterotrophic activity. The estrogenic activity may be lost if aqueous solutions are exposed to ultraviolet light (Smith and Quinn, 1992); this loss of activity is supported by results of previous NTP studies (unpublished, 1986) which indicate that dilutions of 4,4′-diamino-2,2′-stilbenedisulfonic acid exposed to light were unstable. Dilutions were stable for at least 3 hours if protected from light.

An effect which may have been associated with estrogenic activity of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, did occur in the current NTP studies. Cystic endometrial hyperplasia occurred in female mice in the 13-week study; this hyperplasia was most common in the 25,000 ppm dose group (although it also occurred in lower dose groups), was less common in the 50,000 ppm dose group, and was not observed in the 100,000 ppm dose group. However, final mean body weights were significantly lower in the two highest female dose groups than in the control group; ovarian atrophy occurred in all females in the 100,000 ppm dose group and may have been secondary to the marked decrease in body weights. This atrophy may have concealed any cystic endometrial hyperplasia in the 100,000 ppm dose group. It is uncertain whether any estrogenic activity would have been retained in the premixed feed or if the above effect is indeed related to estrogenic activity of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt. Also, there were no apparent estrogenic effects in the NTP 2-year studies. While cystic endometrial hyperplasia occurred in 80% to 91% of all female mice in the 2-year study, it is an extremely common spontaneous lesion of aged female mice and is probably not an appropriate endpoint for determination of an estrogenic effect.

The risk of exposure for the general population is much greater for the derivative dyes and FWAs than for 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt. A review of the available information indicates low toxicity for stilbene derivative FWAs as well. Most stilbene derivative FWAs are water soluble (Gold, 1975). Photochemical and biological degradation occurred in representative water-soluble FWAs; however, the importance of stilbene derivative FWAs in the food chain remains largely undetermined (Zinkernagel, 1975). The highest estimate of total intake of FWAs from contaminated food and from contact with the skin by consumers is 4.4 micrograms/kg per day (Buxtorf, 1975).

Two-year feed studies were conducted in albino rats with the following four FWAs to determine toxic and carcinogenic effects: sodium 2-(4-styryl-3-sulfophenyl)-2H-naphtho[1,2-d]triazole; disodium 4,4′-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2′-disulfonate; disodium 4,4′-bis{[4-anilino-6-(N-methyl-N-2-hydroxyethyl)-amino-1,3,5-triazin-2-yl]amino}stilbene-2,2′-disulfonate; and disodium 4,4′-bis(2-sulfostyryl)biphenyl. Each study had a control group and three treatment groups (0 ppm, 40 ppm, 200 ppm, and 1,000 ppm) of 50 male and 50 female rats. There were no significant toxic or carcinogenic effects in these rat studies (Keplinger *et al.*, 1975a). Daily oral administration of FWAs of the bis(triazinylamino or triazolyl)stilbenedisulfonic acid type at doses up to 1,000 mg/kg to rats from the sixth to the fifteenth day of pregnancy and to rabbits from the sixth to eighteenth day of pregnancy caused no embryotoxic or teratogenic effects (Lorke and Machemer, 1975). No significant adverse effects occurred in three-generation reproductive studies in albino rats with the four FWAs mentioned above at doses of 40, 200, and 1,000 ppm (Keplinger *et al.*, 1975b).

Conclusions

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in male or female F344/N rats receiving 12,500 or 25,000 ppm. There was *no evidence of carcinogenic activity of* 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt in male or female B6C3F$_1$ mice receiving 6,250 or 12,500 ppm.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and public discussion on this Technical Report appears on page 10.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF 4,4′-DIAMINO-2,2′-STILBENEDISULFONIC ACID, DISODIUM SALT

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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	12,500 ppm	25,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	7	6	6
Moribund kills	21	24	20
Survivors			
Terminal sacrifice	22	20	23
Died last week of study			1
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, colon	(47)	(14)	(49)
Intestine large, rectum	(47)	(14)	(48)
Intestine small, duodenum	(48)	(12)	(49)
Intestine small, ileum	(48)	(12)	(48)
Adenoma, papillary	1 (2%)		
Intestine small, jejunum	(47)	(11)	(48)
Liver	(50)	(49)	(50)
Neoplastic nodule	1 (2%)	1 (2%)	4 (8%)
Neoplastic nodule, multiple			1 (2%)
Pheochromocytoma malignant, metastatic, adrenal gland			1 (2%)
Mesentery	(5)	(2)	(5)
Fibrous histiocytoma			1 (20%)
Pancreas	(49)	(16)	(49)
Sarcoma, metastatic, tissue NOS		1 (6%)	
Salivary glands	(50)	(14)	(50)
Stomach, forestomach	(49)	(21)	(49)
Papilloma squamous			1 (2%)
Sarcoma, metastatic, tissue NOS		1 (5%)	
Stomach, glandular	(48)	(21)	(50)
Tongue			(3)
Papilloma squamous			1 (33%)
Cardiovascular System			
Heart	(50)	(20)	(50)
Sarcoma, metastatic, skin		1 (5%)	1 (2%)
Endocrine System			
Adrenal gland, cortex	(50)	(49)	(50)
Adrenal gland, medulla	(48)	(50)	(50)
Pheochromocytoma malignant	2 (4%)	3 (6%)	4 (8%)
Pheochromocytoma malignant, multiple		1 (2%)	4 (8%)
Pheochromocytoma complex			1 (2%)
Pheochromocytoma benign	12 (25%)	14 (28%)	14 (28%)
Pheochromocytoma benign, multiple	4 (8%)	4 (8%)	5 (10%)
Islets, pancreatic	(49)	(16)	(49)
Adenoma			2 (4%)
Carcinoma	1 (2%)		2 (4%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Endocrine System (continued)			
Parathyroid gland	(49)	(15)	(45)
Adenoma	1 (2%)		
Pituitary gland	(50)	(49)	(50)
Pars distalis, adenoma	16 (32%)	8 (16%)	10 (20%)
Pars distalis, adenoma, multiple	1 (2%)		
Thyroid gland	(49)	(18)	(50)
C-cell, adenoma	2 (4%)	1 (6%)	
C-cell, carcinoma	2 (4%)	1 (6%)	3 (6%)
General Body System			
Tissue NOS		(1)	
Sarcoma		1 (100%)	
Genital System			
Epididymis	(50)	(13)	(50)
Preputial gland	(50)	(20)	(49)
Adenoma	8 (16%)	4 (20%)	4 (8%)
Carcinoma	1 (2%)	2 (10%)	1 (2%)
Fibrosarcoma			1 (2%)
Papilloma squamous			1 (2%)
Bilateral, carcinoma	2 (4%)	1 (5%)	3 (6%)
Prostate	(50)	(14)	(49)
Seminal vesicle	(48)	(37)	(47)
Testes	(49)	(50)	(50)
Interstitial cell, adenoma	4 (8%)	9 (18%)	3 (6%)
Interstitial cell, adenoma, multiple	37 (76%)	31 (62%)	43 (86%)
Testes, glandular	(1)		
Interstitial cell, adenoma, multiple	1 (100%)		
Hematopoietic System			
Bone marrow	(49)	(14)	(50)
Pheochromocytoma malignant, metastatic, adrenal gland			1 (2%)
Lymph node	(50)	(33)	(50)
Deep cervical, carcinoma, metastatic, thyroid gland	1 (2%)		
Inguinal, renal, iliac, bronchial, mediastinal, sarcoma, metastatic, skin			1 (2%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung		1 (3%)	
Lymph node, mesenteric	(50)	(18)	(50)
Spleen	(49)	(49)	(50)
Histiocytic sarcoma		1 (2%)	
Pheochromocytoma malignant, metastatic, adrenal gland			1 (2%)
Thymus	(50)	(14)	(46)
Sarcoma, metastatic, skin			1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Integumentary System			
Mammary gland	(48)	(18)	(50)
Adenocarcinoma		1 (6%)	
Fibroadenoma	3 (6%)	2 (11%)	4 (8%)
Skin	(49)	(21)	(50)
Keratoacanthoma	2 (4%)	2 (10%)	2 (4%)
Papilloma squamous	1 (2%)		1 (2%)
Squamous cell carcinoma	1 (2%)	1 (5%)	
Trichoepithelioma			2 (4%)
Sebaceous gland, adenoma		1 (5%)	
Subcutaneous tissue, fibroma		1 (5%)	1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)		2 (4%)
Subcutaneous tissue, lipoma			1 (2%)
Subcutaneous tissue, sarcoma		2 (10%)	1 (2%)
Subcutaneous tissue, schwannoma benign	1 (2%)		
Musculoskeletal System			
Skeletal muscle		(1)	(1)
Diaphragm, sarcoma, metastatic, tissue NOS		1 (100%)	
Nervous System			
Brain	(50)	(13)	(50)
Astrocytoma malignant			1 (2%)
Sarcoma	1 (2%)		
Respiratory System			
Larynx	(26)		(27)
Carcinoma, metastatic, thyroid gland	1 (4%)		
Lung	(50)	(30)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (3%)	2 (4%)
Alveolar/bronchiolar carcinoma	1 (2%)	1 (3%)	
Alveolar/bronchiolar carcinoma, multiple			1 (2%)
Carcinoma, metastatic, preputial gland		1 (3%)	
Carcinoma, metastatic, thyroid gland	1 (2%)		
Pheochromocytoma malignant, metastatic, adrenal gland			1 (2%)
Sarcoma, metastatic, skin		1 (3%)	1 (2%)
Squamous cell carcinoma, metastatic, Zymbal's gland		1 (3%)	
Mediastinum, alveolar/bronchiolar carcinoma			1 (2%)
Mediastinum, hemangiosarcoma		1 (3%)	
Nose	(49)	(14)	(50)
Trachea	(50)	(14)	(50)
Carcinoma, metastatic, thyroid gland	1 (2%)		
Sarcoma, metastatic, skin		1 (7%)	1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Special Senses System			
Ear	(1)		
External ear, squamous cell carcinoma	1 (100%)		
Zymbal's gland		(1)	(1)
Adenoma			1 (100%)
Squamous cell carcinoma		1 (100%)	
Urinary System			
Kidney	(50)	(45)	(50)
Renal tubule, adenoma			1 (2%)
Urinary bladder	(50)	(14)	(49)
Transitional epithelium, papilloma	1 (2%)		
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	
Leukemia mononuclear	23 (46%)	29 (58%)	28 (56%)
Mesothelioma benign		1 (2%)	
Mesothelioma malignant	3 (6%)	1 (2%)	3 (6%)
Tumor Summary			
Total animals with primary neoplasms[c]	50	50	50
Total primary neoplasms	136	127	161
Total animals with benign neoplasms	47	44	48
Total benign neoplasms	97	80	104
Total animals with malignant neoplasms	32	38	42
Total malignant neoplasms	39	47	57
Total animals with secondary neoplasms	1	4	2
Total secondary neoplasms	4	9	9

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except secondary tumors

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm

Number of Days on Study	067	379	443	446	540	561	562	571	579	580	606	620	624	624	628	630	640	641	648	662	687	692	692	696	704
Carcass ID Number	0101	0081	0095	0032	0031	0033	0071	0045	0052	0044	0125	0054	0051	0053	0043	0104	0112	0121	0034	0022	0062	0075	0094	0092	0083
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, papillary																						X			
Intestine small, jejunum	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Mesentery														+		+						+			
Pancreas	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	A	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Tooth																							+		
Cardiovascular System																									
Blood vessel	+																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign					X										X	X	X		X				X		X
Pheochromocytoma benign, multiple																									
Islets, pancreatic	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																					X				
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X		X		X	X		X								X						X	
Pars distalis, adenoma, multiple																									
Thyroid gland	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																								X	
C-cell, carcinoma																									

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

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	704	708	713	729	729	729	732	732	732	732	732	732	733	733	733	733	733	733	734	734	734	734	734	734		
Carcass ID Number	011	012	006	042	047	093	091	015	061	073	084	085	012	013	024	064	065	082	123	012	014	041	047	073	075	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma, papillary																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule																									X	1
Mesentery								+																+		5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tooth																										1
Cardiovascular System																										
Blood vessel				+																						2
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	48
Pheochromocytoma malignant			X																			X				2
Pheochromocytoma benign														X		X			X			X	X			12
Pheochromocytoma benign, multiple	X	X						X																X		4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	49
Adenoma										X																1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma			X				X			X		X	X		X						X	X	X			16
Pars distalis, adenoma, multiple	X																									1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma																			X							2
C-cell, carcinoma	X							X																		2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	067	379	443	446	540	561	562	571	579	580	606	620	624	624	628	630	640	641	648	662	687	692	692	696	704
Carcass ID Number	0101	0081	0095	0032	0031	0033	0071	0045	0052	0044	0125	0054	0051	0053	0043	0140	0112	0121	0034	0022	0062	0075	0094	0092	0083
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X					X											X				
Carcinoma																									
Bilateral, carcinoma																								X	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma					X		X	X																X	
Interstitial cell, adenoma, multiple				X		X			X			X		X		X	X		X	X	X	X	X		X
Testes, glandular															+										
Interstitial cell, adenoma, multiple															X										
Hematopoietic System																									
Blood																						+			
Bone marrow	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deep cervical, carcinoma, metastatic, thyroid gland																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+
Fibroadenoma	X														X										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Keratoacanthoma																X									
Papilloma squamous																									
Squamous cell carcinoma																									
Subcutaneous tissue, fibrosarcoma																X									
Subcutaneous tissue, schwannoma benign				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	704	708	713	729	729	729	732	732	732	732	732	732	732	733	733	733	733	733	733	734	734	734	734	734	734	
Carcass ID Number	0011112	0012111	0063113	0042132	0074124	0093113	0011231	0011515	0061321	0067134	0088245	0081222	0010123	0010134	0060244	0066245	0068232	0012313	0010232	0011214	0040141	0047122	0140123	0110125		**Total Tissues/ Tumors**
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X			X				X			X		X						8
Carcinoma							X																			1
Bilateral, carcinoma														X												2
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Interstitial cell, adenoma																										4
Interstitial cell, adenoma, multiple	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	37
Testes, glandular																										1
Interstitial cell, adenoma, multiple																										1
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Deep cervical, carcinoma, metastatic, thyroid gland	X																									1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibroadenoma																		X								3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Keratoacanthoma											X															2
Papilloma squamous																									X	1
Squamous cell carcinoma												X														1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, schwannoma benign																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	067	379	443	446	540	561	562	571	579	580	606	620	624	624	628	630	640	641	648	662	687	692	692	696	704
Carcass ID Number	101	081	095	093	093	093	097	094	095	094	125	054	051	053	043	040	112	121	034	022	026	075	094	092	083
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma		X																							
Respiratory System																										
Larynx																									+
Carcinoma, metastatic, thyroid gland																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, thyroid gland																									
Nose	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Special Senses System																									
Ear																									
External ear, squamous cell carcinoma																									
Eye																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X	X				X	X	X	X			X	X		X		X	X	X	X
Mesothelioma malignant																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	704	708	713	729	729	729	732	732	732	732	732	732	732	733	733	733	733	733	733	734	734	734	734	734	734	734
Carcass ID Number	0011	0021	0063	0042	0074	0093	0111	0115	0061	0073	0084	0085	0092	0101	0093	0094	0096	0095	0098	0123	0101	0094	0094	0097	0101	0105	**Total Tissues/ Tumors**
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma																											1
Respiratory System																											
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	26
Carcinoma, metastatic, thyroid gland	X																										1
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma														X													1
Alveolar/bronchiolar carcinoma																				X							1
Carcinoma, metastatic, thyroid gland	X																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, thyroid gland	X																										1
Special Senses System																											
Ear														+													1
External ear, squamous cell carcinoma														X													1
Eye																				+				+			2
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma													X														1
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X	X			X				X		X	X					X				X		X	X		23
Mesothelioma malignant								X					X											X			3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm

Number of Days on Study	134	327	510	521	522	571	580	583	595	609	615	618	621	623	644	653	658	663	663	663	676	683	683	687	697
Carcass ID Number	021	028	032	031	034	031	033	035	031	033	029	030	026	027	025	055	062	093	034	036	074	030	034	043	026
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+										+	
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+										+	
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Intestine small	+	+	+	A	+	+	+	+	+	+	+	A	+	+											
Intestine small, duodenum	+	+	+	A	+	+	+	+	+	+	+	A	+	+											
Intestine small, ileum	+	+	+	A	+	+	+	+	+	+	+	A	+	+											
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	+	+	A	+	A											
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Mesentery																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Sarcoma, metastatic, tissue NOS																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+		+		+	
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+		+		+	
Sarcoma, metastatic, tissue NOS																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+		+		+	
Cardiovascular System																									
Blood vessel														+											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+										+
Sarcoma, metastatic, skin											X														
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																			X						
Pheochromocytoma malignant, multiple																									
Pheochromocytoma benign								X						X			X			X	X			X	X
Pheochromocytoma benign, multiple																							X		
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Parathyroid gland	+	M	+	+	+	+	M	+	+	+	+	+	+	+											
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X		X									X	X										X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
C-cell, adenoma																									
C-cell, carcinoma																									
General Body System																									
Tissue NOS																									
Sarcoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	697	719	725	725	725	729	729	729	732	732	732	732	732	732	733	733	733	733	733	733	734	734	734	734	734	
Carcass ID Number	0282	0273	0284	0353	0364	0272	0304	0335	0253	0262	0264	0301	0324	0365	0252	0285	0305	0321	0325	0334	0225	0255	0292	0351	0354	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus		+																								15
Intestine large																										15
Intestine large, cecum																										15
Intestine large, colon																										14
Intestine large, rectum																										14
Intestine small																										12
Intestine small, duodenum																										12
Intestine small, ileum																										12
Intestine small, jejunum																										11
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	49
Neoplastic nodule										X																1
Mesentery				+										+												2
Pancreas		+				+																				16
Sarcoma, metastatic, tissue NOS		X																								1
Salivary glands																										14
Stomach	+	+																								21
Stomach, forestomach	+	+																								21
Sarcoma, metastatic, tissue NOS		X																								1
Stomach, glandular	+	+																								21
Cardiovascular System																										
Blood vessel																										1
Heart				+							+										+			+		20
Sarcoma, metastatic, skin																										1
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	49
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant	X																						X			3
Pheochromocytoma malignant, multiple																			X							1
Pheochromocytoma benign	X		X						X					X		X				X				X		14
Pheochromocytoma benign, multiple					X										X							X				4
Islets, pancreatic		+				+																				16
Parathyroid gland				+															+			+				15
Pituitary gland	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma		X								X	X															8
Thyroid gland				+				+								+						+				18
C-cell, adenoma																						X				1
C-cell, carcinoma								X																		1
General Body System																										
Tissue NOS		+																								1
Sarcoma		X																								1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	134	327	510	521	521	571	780	883	995	609	615	618	621	623	644	653	658	663	663	663	676	683	683	687	697
Carcass ID Number	029	028	032	031	034	031	031	035	031	033	029	030	026	027	025	035	036	029	034	036	027	030	034	033	026
	1	1	3	1	5	5	4	5	2	3	4	2	1	1	4	2	2	3	3	3	4	3	4	4	3
Genital System																									
Epididymis	+	+	+	+	+	+		+	+	+	+	+	+	+											
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+			+				+
Adenoma							X		X									X							
Carcinoma																					X				X
Bilateral, carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Seminal vesicle	+	+	+	A	+	+	+	+	+	+	+	+	+	+		+	A	+	+	+	+	+		+	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma			X	X				X						X		X				X		X			
Interstitial cell, adenoma, multiple										X	X				X		X	X			X		X	X	X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+							+			+	+
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																									X
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+										+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								X	
Thymus	+	+	+	+	M	+	+	+	M	+	+	+	+	+											
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Adenocarcinoma																									
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+								+
Keratoacanthoma																	X								
Squamous cell carcinoma																									
Sebaceous gland, adenoma																									
Subcutaneous tissue, fibroma									X																
Subcutaneous tissue, sarcoma											X						X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Skeletal muscle																									
Diaphragm, sarcoma, metastatic, tissue NOS																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+											

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	697	719	725	725	725	729	729	729	732	732	732	732	732	732	733	733	733	733	733	733	734	734	734	734	734	
Carcass ID Number	0282	0273	0284	0353	0364	0272	0334	0335	0325	0322	0326	0301	0324	0326	0252	0258	0305	0321	0325	0341	0251	0255	0292	0351	0354	**Total Tissues/ Tumors**
Genital System																										
Epididymis																										13
Preputial gland											+												+	+		20
Adenoma																								X		4
Carcinoma																										2
Bilateral, carcinoma											X															1
Prostate																										14
Seminal vesicle	+	+				+	+			+	+	+	+	+	+	+		+	+	+	+			+	+	37
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma			X							X																9
Interstitial cell, adenoma, multiple	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	31
Hematopoietic System																										
Bone marrow																										14
Lymph node			+	+	+	+	+			+	+	+		+	+	+		+	+	+	+				+	33
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Lymph node, mesenteric				+								+							+							18
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Thymus				+	+																					14
Integumentary System																										
Mammary gland				+			+			+	+															18
Adenocarcinoma				X																						1
Fibroadenoma							X			X																2
Skin					+				+	+								+	+							21
Keratoacanthoma																			X							2
Squamous cell carcinoma																		X								1
Sebaceous gland, adenoma									X																	1
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, sarcoma																										2
Musculoskeletal System																										
Bone																										14
Skeletal muscle		+																								1
Diaphragm, sarcoma, metastatic, tissue NOS		X																								1
Nervous System																										
Brain																										13

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	134	327	510	521	521	571	580	583	595	609	615	618	621	623	644	653	658	663	663	663	676	683	683	687	697
Carcass ID Number	0291	0281	0323	0331	0343	0351	0341	0355	0312	0333	0294	0302	0361	0271	0254	0235	0262	0293	0343	0363	0274	0303	0344	0343	0263
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+	+			+			+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									X
Carcinoma, metastatic, preputial gland																									X
Sarcoma, metastatic, skin											X														
Squamous cell carcinoma, metastatic, Zymbal's gland										X															
Mediastinum, hemangiosarcoma	X																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Sarcoma, metastatic, skin											X														
Special Senses System																									
Eye			+																						
Zymbal's gland										+															
Squamous cell carcinoma										X															
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+		+	+	+	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+											
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																								X	
Leukemia mononuclear			X		X	X		X	X			X	X		X	X		X	X	X	X	X	X		X
Mesothelioma benign									X																
Mesothelioma malignant									X																

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	697	719	725	725	725	729	729	729	732	732	732	732	732	732	733	733	733	733	733	733	734	734	734	734	734	
Carcass ID Number	0282	0273	0284	0353	0364	0272	0304	0335	0253	0262	0264	0301	0324	0365	0252	0258	0305	0221	0255	0341	0251	0255	0292	0351	0354	**Total Tissues/ Tumors**
Respiratory System																										
Lung					+	+		+		+	+			+		+	+	+		+					+	30
Alveolar/bronchiolar adenoma																	X									1
Alveolar/bronchiolar carcinoma																										1
Carcinoma, metastatic, preputial gland																										1
Sarcoma, metastatic, skin																										1
Squamous cell carcinoma, metastatic, Zymbal's gland																										1
Mediastinum, hemangiosarcoma																										1
Nose																										14
Trachea																										14
Sarcoma, metastatic, skin																										1
Special Senses System																										
Eye																										1
Zymbal's gland																										1
Squamous cell carcinoma																										1
Urinary System																										
Kidney	+	+	+	+		+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Urinary bladder																										14
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Leukemia mononuclear	X		X	X	X			X		X			X	X		X	X	X		X				X		29
Mesothelioma benign																										1
Mesothelioma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm

Number of Days on Study	491	508	520	538	551	555	561	663	665	658	688	613	624	635	644	644	658	663	676	676	683	697	700	704	718
Carcass ID Number	0571	0522	0585	0603	0514	0555	0582	0494	0442	0512	0575	0533	0523	0572	0541	0545	0574	0594	0524	0593	0534	0561	0591	0542	0595
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Neoplastic nodule, multiple																									
Pheochromocytoma malignant, metastatic, adrenal gland																									
Mesentery																						+	+		
Fibrous histiocytoma																						X			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous													X												
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Papilloma squamous																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin	X																								
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																	X								X
Pheochromocytoma malignant, multiple																					X			X	
Pheochromocytoma complex			X																						
Pheochromocytoma benign			X										X					X	X						
Pheochromocytoma benign, multiple																X				X					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Adenoma																									
Carcinoma													X												
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	M	+	+	+	M	+	+	+	M	+	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma													X		X			X				X			X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, carcinoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	720	729	729	729	732	732	732	732	732	732	733	733	733	733	733	733	734	734	734	734	734	734	734		
Carcass ID Number	0532	0491	0501	0594	0493	0502	0503	0504	0505	0552	0604	0552	0553	0543	0544	0564	0583	0601	0531	0554	0563	0581	0592	0602	0605	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule												X			X	X	X									4
Neoplastic nodule, multiple	X																									1
Pheochromocytoma malignant, metastatic, adrenal gland																					X					1
Mesentery						+													+				+			5
Fibrous histiocytoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Papilloma squamous																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue	+			+															+							3
Papilloma squamous				X																						1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, skin																										1
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant					X										X											4
Pheochromocytoma malignant, multiple																	X				X					4
Pheochromocytoma complex																										1
Pheochromocytoma benign		X					X	X	X	X	X			X	X								X	X		14
Pheochromocytoma benign, multiple	X												X												X	5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																		X							X	2
Carcinoma												X														2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma		X					X			X							X	X								10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, carcinoma			X				X									X										3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	7	7	7
	9	0	2	3	5	5	6	6	6	8	8	1	2	3	4	4	5	6	7	7	8	9	0	0	1
	1	8	0	8	1	5	1	3	5	8	8	3	4	5	4	4	8	3	6	6	3	7	0	4	8
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	5	5	6	5	5	5	4	4	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5
	7	2	8	0	1	5	8	9	9	1	7	3	2	7	4	4	7	9	2	9	3	6	9	4	9
	1	2	5	3	4	5	2	4	2	2	5	3	3	2	1	5	4	5	4	3	4	1	1	2	5
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma							X																		
Carcinoma																									
Fibrosarcoma																									
Papilloma squamous																									
Bilateral, carcinoma																			X			X			
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	A	+	+	+	+	M	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma			X																					X	
Interstitial cell, adenoma, multiple		X			X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant, metastatic, adrenal gland																									
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inguinal, renal, iliac, bronchial, mediastinal, sarcoma, metastatic, skin	X																								
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant, metastatic, adrenal gland																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Sarcoma, metastatic, skin	X																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	720	729	729	729	732	732	732	732	732	732	732	733	733	733	733	733	733	733	734	734	734	734	734	734	734	
Carcass ID Number	0532	0491	0501	0594	0493	0502	0503	0504	0505	0552	0604	0525	0553	0543	0544	0564	0583	0601	0531	0554	0563	0581	0592	0602	0605	**Total Tissues/ Tumors**
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma						X					X												X			4
Carcinoma	X																									1
Fibrosarcoma													X													1
Papilloma squamous														X												1
Bilateral, carcinoma																	X									3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma							X																			3
Interstitial cell, adenoma, multiple	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	43
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant, metastatic, adrenal gland																					X					1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Inguinal, renal, iliac, bronchial, mediastinal, sarcoma, metastatic, skin																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant, metastatic, adrenal gland																					X					1
Thymus	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Sarcoma, metastatic, skin																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	491	508	520	538	551	555	561	663	665	688	688	613	624	635	644	644	658	663	676	677	683	697	700	704	718
Carcass ID Number	057	052	058	060	051	055	058	044	049	051	057	053	053	057	055	055	057	049	052	059	053	056	059	054	059
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																							X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma														X											
Papilloma squamous																									
Trichoepithelioma											X								X						
Subcutaneous tissue, fibroma																						X			
Subcutaneous tissue, fibrosarcoma										X															
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, sarcoma	X																								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																	X								
Respiratory System																									
Larynx																								+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X						
Alveolar/bronchiolar carcinoma, multiple																									
Pheochromocytoma malignant, metastatic, adrenal gland																									
Sarcoma, metastatic, skin	X																								
Mediastinum, alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skin	X																								
Special Senses System																									
Eye																									
Zymbal's gland																									
Adenoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	720	729	729	729	732	732	732	732	732	732	732	733	733	733	733	733	733	733	734	734	734	734	734	734	734	
Carcass ID Number	053 2	049 1	050 1	059 4	049 3	050 2	050 3	050 4	050 5	055 2	060 4	055 5	055 3	054 3	054 4	056 4	058 3	060 1	053 1	055 4	056 3	058 1	059 2	060 2	060 5	**Total Tissues/ Tumors**
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma	X									X					X											4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																									X	2
Papilloma squamous																			X							1
Trichoepithelioma																										2
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, fibrosarcoma				X																						2
Subcutaneous tissue, lipoma																						X				1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																							+			1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	27
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X																				2
Alveolar/bronchiolar carcinoma, multiple												X														1
Pheochromocytoma malignant, metastatic, adrenal gland																					X					1
Sarcoma, metastatic, skin																										1
Mediastinum, alveolar/bronchiolar carcinoma												X														1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, skin																										1
Special Senses System																										
Eye	+						+																			2
Zymbal's gland							+																			1
Adenoma							X																			1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	491	508	520	538	551	555	561	663	665	688	688	613	624	635	644	644	658	663	676	677	683	697	700	704	718
Carcass ID Number	0571	0552	0585	0603	0514	0555	0558	0544	0549	0512	0557	0533	0532	0557	0544	0545	0547	0549	0524	0553	0533	0561	0591	0542	0559
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X	X	X	X	X	X	X	X			X		X	X	X		X		X	X			X	X
Mesothelioma malignant																							X		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	720	729	729	729	732	732	732	732	732	732	732	733	733	733	733	733	733	733	734	734	734	734	734	734	734	
Carcass ID Number	053	049	051	059	049	052	053	054	055	055	060	052	053	054	044	046	058	060	053	055	056	058	059	060	065	Total Tissues/ Tumors
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																					X					1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X			X	X		X				X				X	X		X	X	X				28
Mesothelioma malignant																			X				X			3

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm	12,500 ppm	25,000 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rates[a]	16/48 (33%)	18/50 (36%)	19/50 (38%)
Adjusted rates[b]	50.5%	56.0%	59.6%
Terminal rates[c]	7/21 (33%)	7/20 (35%)	12/24 (50%)
First incidence (days)	540	583	520
Life table tests[d]	P=0.441	P=0.405	P=0.477
Logistic regression tests[d]	P=0.396	P=0.491	P=0.438
Cochran-Armitage test[d]	P=0.354		
Fisher exact test[d]		P=0.474	P=0.393
Adrenal Medulla: Malignant Pheochromocytoma			
Overall rates	2/48 (4%)	4/50 (8%)	8/50 (16%)
Adjusted rates	8.9%	16.1%	27.6%
Terminal rates	1/21 (5%)	2/20 (10%)	4/24 (17%)
First incidence (days)	713	663	658
Life table tests	P=0.049	P=0.339	P=0.074
Logistic regression tests	P=0.037	P=0.358	P=0.059
Cochran-Armitage test	P=0.033		
Fisher exact test		P=0.359	P=0.053
Adrenal Medulla: Pheochromocytoma (Benign, Complex, or Malignant)			
Overall rates	17/48 (35%)	21/50 (42%)	26/50 (52%)
Adjusted rates	52.7%	63.9%	73.6%
Terminal rates	7/21 (33%)	9/20 (45%)	15/24 (63%)
First incidence (days)	540	583	520
Life table tests	P=0.134	P=0.280	P=0.150
Logistic regression tests	P=0.067	P=0.334	P=0.084
Cochran-Armitage test	P=0.060		
Fisher exact test		P=0.323	P=0.073
Liver: Neoplastic Nodule			
Overall rates	1/50 (2%)	1/49 (2%)	5/50 (10%)
Adjusted rates	4.5%	5.3%	20.0%
Terminal rates	1/22 (5%)	1/19 (5%)	4/24 (17%)
First incidence (days)	729 (T)	729 (T)	720
Life table tests	P=0.063	P=0.730	P=0.124
Logistic regression tests	P=0.058	P=0.730	P=0.118
Cochran-Armitage test	P=0.049		
Fisher exact test		P=0.747	P=0.102
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	2/50 (4%)	2/30 (7%)	3/50 (6%)
Adjusted rates	9.1%	13.6%	11.2%
Terminal rates	2/22 (9%)	1/10 (10%)	2/24 (8%)
First incidence (days)	729 (T)	697	676
Life table tests	P=0.454	P=0.469	P=0.538
Logistic regression tests	P=0.441	P=0.399	P=0.527
Cochran-Armitage test	P=0.412		
Fisher exact test		P=0.483	P=0.500

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Mammary Gland: Fibroadenoma			
Overall rates	3/50 (6%)	2/50 (4%)	4/50 (8%)
Adjusted rates	9.1%	10.0%	15.1%
Terminal rates	1/22 (5%)	2/20 (10%)	2/24 (8%)
First incidence (days)	67	729 (T)	700
Life table tests	P=0.456	P=0.526N	P=0.537
Logistic regression tests	P=0.395	P=0.518N	P=0.437
Cochran-Armitage test	P=0.417		
Fisher exact test		P=0.500N	P=0.500
Mammary Gland: Fibroadenoma or Adenocarcinoma			
Overall rates	3/50 (6%)	3/50 (6%)	4/50 (8%)
Adjusted rates	9.1%	13.9%	15.1%
Terminal rates	1/22 (5%)	2/20 (10%)	2/24 (8%)
First incidence (days)	67	725	700
Life table tests	P=0.463	P=0.642	P=0.537
Logistic regression tests	P=0.407	P=0.653	P=0.437
Cochran-Armitage test	P=0.421		
Fisher exact test		P=0.661N	P=0.500
Pancreatic Islets: Adenoma or Carcinoma			
Overall rates	1/49 (2%)	0/16 (0%)[e]	4/49 (8%)
Adjusted rates	3.3%		14.8%
Terminal rates	0/22 (0%)		3/24 (13%)
First incidence (days)	687		624
Life table tests			P=0.196
Logistic regression tests			P=0.186
Fisher exact test			P=0.181
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	17/50 (34%)	8/49 (16%)	10/50 (20%)
Adjusted rates	51.2%	24.6%	32.5%
Terminal rates	8/22 (36%)	2/20 (10%)	5/24 (21%)
First incidence (days)	443	327	624
Life table tests	P=0.059N	P=0.056N	P=0.076N
Logistic regression tests	P=0.061N	P=0.037N	P=0.077N
Cochran-Armitage test	P=0.063N		
Fisher exact test		P=0.036N	P=0.088N
Preputial Gland: Adenoma			
Overall rates	8/50 (16%)	4/20 (20%)[e]	4/49 (8%)
Adjusted rates	28.7%		14.5%
Terminal rates	5/22 (23%)		3/24 (13%)
First incidence (days)	540		561
Life table tests			P=0.156N
Logistic regression tests			P=0.169N
Fisher exact test			P=0.188N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Preputial Gland: Carcinoma			
Overall rates	3/50 (6%)	3/20 (15%)[e]	4/49 (8%)
Adjusted rates	12.5%		13.9%
Terminal rates	2/22 (9%)		1/24 (4%)
First incidence (days)	696		676
Life table tests			P=0.548
Logistic regression tests			P=0.526
Fisher exact test			P=0.489
Preputial Gland: Adenoma or Carcinoma			
Overall rates	11/50 (22%)	7/20 (35%)[e]	8/49 (16%)
Adjusted rates	39.4%		26.9%
Terminal rates	7/22 (32%)		4/24 (17%)
First incidence (days)	540		561
Life table tests			P=0.258N
Logistic regression tests			P=0.279N
Fisher exact test			P=0.323N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rates	2.9%	2.4%	9.7%
Terminal rates	0/22 (0%)	0/20 (0%)	1/24 (4%)
First incidence (days)	630	595	588
Life table tests	P=0.220	P=0.752N	P=0.333
Logistic regression tests	P=0.194	P=0.758	P=0.303
Cochran-Armitage test	P=0.202		
Fisher exact test		P=0.753N	P=0.309
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma			
Overall rates	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rates	2.9%	5.4%	8.4%
Terminal rates	0/22 (0%)	0/20 (0%)	1/24 (4%)
First incidence (days)	630	615	491
Life table tests	P=0.243	P=0.517	P=0.331
Logistic regression tests	P=0.191	P=0.497	P=0.253
Cochran-Armitage test	P=0.222		
Fisher exact test		P=0.500	P=0.309
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma			
Overall rates	1/50 (2%)	3/50 (6%)	4/50 (8%)
Adjusted rates	2.9%	7.6%	11.5%
Terminal rates	0/22 (0%)	0/20 (0%)	1/24 (4%)
First incidence (days)	630	595	491
Life table tests	P=0.153	P=0.326	P=0.208
Logistic regression tests	P=0.111	P=0.299	P=0.151
Cochran-Armitage test	P=0.133		
Fisher exact test		P=0.309	P=0.181

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Testes: Adenoma			
Overall rates	42/50 (84%)	40/50 (80%)	46/50 (92%)
Adjusted rates	97.6%	97.5%	100.0%
Terminal rates	21/22 (95%)	19/20 (95%)	24/24 (100%)
First incidence (days)	446	510	508
Life table tests	P=0.466	P=0.538N	P=0.491
Logistic regression tests	P=0.252	P=0.293N	P=0.307
Cochran-Armitage test	P=0.161		
Fisher exact test		P=0.398N	P=0.178
Thyroid Gland (C-cell): Carcinoma			
Overall rates	2/49 (4%)	1/18 (6%)[e]	3/50 (6%)
Adjusted rates	8.2%		12.5%
Terminal rates	1/22 (5%)		3/24 (13%)
First incidence (days)	704		729 (T)
Life table tests			P=0.535
Logistic regression tests			P=0.534
Fisher exact test			P=0.510
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rates	4/49 (8%)	2/18 (11%)[e]	3/50 (6%)
Adjusted rates	15.8%		12.5%
Terminal rates	2/22 (9%)		3/24 (13%)
First incidence (days)	696		729 (T)
Life table tests			P=0.458N
Logistic regression tests			P=0.464N
Fisher exact test			P=0.489N
All Organs: Mononuclear Cell Leukemia			
Overall rates	23/50 (46%)	29/50 (58%)	28/50 (56%)
Adjusted rates	60.6%	71.1%	64.7%
Terminal rates	8/22 (36%)	9/20 (45%)	10/24 (42%)
First incidence (days)	561	510	508
Life table tests	P=0.335	P=0.207	P=0.350
Logistic regression tests	P=0.197	P=0.165	P=0.218
Cochran-Armitage test	P=0.184		
Fisher exact test		P=0.158	P=0.212
All Organs: Benign or Malignant Mesothelioma			
Overall rates	3/50 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rates	13.6%	2.4%	11.6%
Terminal rates	3/22 (14%)	0/20 (0%)	2/24 (8%)
First incidence (days)	729 (T)	595	700
Life table tests	P=0.561N	P=0.327N	P=0.624N
Logistic regression tests	P=0.575N	P=0.299N	P=0.631N
Cochran-Armitage test	P=0.594		
Fisher exact test		P=0.309N	P=0.661N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
All Organs: Benign Tumors			
Overall rates	47/50 (94%)	44/50 (88%)	48/50 (96%)
Adjusted rates	100.0%	97.7%	100.0%
Terminal rates	22/22 (100%)	19/20 (95%)	24/24 (100%)
First incidence (days)	67	327	508
Life table tests	P=0.416N	P=0.474N	P=0.446N
Logistic regression tests	P=0.572N	P=0.205N	P=0.682
Cochran-Armitage test	P=0.424		
Fisher exact test		P=0.243N	P=0.500
All Organs: Malignant Tumors			
Overall rates	32/50 (64%)	38/50 (76%)	42/50 (84%)
Adjusted rates	77.6%	82.4%	85.6%
Terminal rates	13/22 (59%)	12/20 (60%)	17/24 (71%)
First incidence (days)	379	134	491
Life table tests	P=0.170	P=0.214	P=0.179
Logistic regression tests	P=0.017	P=0.143	P=0.025
Cochran-Armitage test	P=0.014		
Fisher exact test		P=0.138	P=0.020
All Organs: Benign or Malignant Tumors			
Overall rates	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	22/22 (100%)	20/20 (100%)	24/24 (100%)
First incidence (days)	67	134	491
Life table tests	P=0.364N	P=0.473	P=0.392N
Logistic regression tests	–[f]	–	–
Cochran-Armitage test	–		
Fisher exact test		P=1.000N	P=1.000N

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus, statistical comparisons with the controls are not appropriate.
[f] Value of statistic cannot be computed

TABLE A4
Historical Incidence of Pheochromocytomas of the Adrenal Medulla in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Benign Pheochromocytoma	Malignant Pheochromocytoma	Benign or Malignant Pheochromocytoma
Overall Historical Incidence			
Total	284/788 (36.0%)	39/788 (4.9%)	306/788[b] (38.8%)
Standard deviation	9.3%	5.8%	8.4%
Range	14%–47%	0%–20%	22%–48%

[a] Data as of 29 March 1991
[b] Includes one complex pheochromocytoma

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Endocrine System			
Adrenal gland, cortex	(50)	(49)	(50)
Angiectasis	1 (2%)		
Hematopoietic cell proliferation	1 (2%)		
Hyperplasia	1 (2%)	1 (2%)	
Inflammation, subacute		1 (2%)	
Necrosis, focal			1 (2%)
Thrombus	1 (2%)		
Vacuolization cytoplasmic, focal	2 (4%)	2 (4%)	1 (2%)
Adrenal gland, medulla	(48)	(50)	(50)
Hyperplasia	17 (35%)	21 (42%)	13 (26%)
Islets, pancreatic	(49)	(16)	(49)
Hyperplasia	4 (8%)	1 (6%)	3 (6%)
Parathyroid gland	(49)	(15)	(45)
Hyperplasia	3 (6%)	1 (7%)	1 (2%)
Pituitary gland	(50)	(49)	(50)
Pars distalis, angiectasis	3 (6%)	3 (6%)	2 (4%)
Pars distalis, cyst	1 (2%)	2 (4%)	7 (14%)
Pars distalis, hyperplasia	3 (6%)	9 (18%)	8 (16%)
Pars nervosa, ectopic tissue		1 (2%)	1 (2%)
Thyroid gland	(49)	(18)	(50)
Cyst	2 (4%)		
C-cell, hyperplasia	5 (10%)		2 (4%)
General Body System			
None			
Genital System			
Preputial gland	(50)	(20)	(49)
Cyst	1 (2%)		
Hyperplasia	1 (2%)		2 (4%)
Inflammation, acute	11 (22%)	2 (10%)	8 (16%)
Inflammation, chronic	1 (2%)		
Prostate	(50)	(14)	(49)
Hyperplasia, focal	6 (12%)		5 (10%)
Inflammation, acute	17 (34%)	1 (7%)	8 (16%)
Inflammation, chronic	2 (4%)		
Seminal vesicle	(48)	(37)	(47)
Atrophy	25 (52%)	15 (41%)	25 (53%)
Inflammation, acute			1 (2%)
Testes	(49)	(50)	(50)
Atrophy	15 (31%)	14 (28%)	24 (48%)
Interstitial cell, hyperplasia	2 (4%)	5 (10%)	2 (4%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Hematopoietic System			
Bone marrow	(49)	(14)	(50)
Fibrosis	1 (2%)		
Hyperplasia	2 (4%)	5 (36%)	2 (4%)
Lymph node	(50)	(33)	(50)
Axillary, hyperplasia, lymphoid		1 (3%)	
Iliac, cyst		1 (3%)	
Inguinal, cyst	1 (2%)		1 (2%)
Inguinal, hyperplasia, lymphoid	2 (4%)	1 (3%)	2 (4%)
Mandibular, cyst	7 (14%)		3 (6%)
Mandibular, hemorrhage			1 (2%)
Mandibular, hyperplasia, lymphoid		1 (3%)	2 (4%)
Mediastinal, cyst	1 (2%)		
Mediastinal, hemorrhage	9 (18%)	8 (24%)	10 (20%)
Mediastinal, pigmentation			3 (6%)
Pancreatic, hemorrhage			1 (2%)
Renal, hemorrhage		1 (3%)	
Renal, inflammation, chronic			1 (2%)
Lymph node, mesenteric	(50)	(18)	(50)
Cyst	1 (2%)	2 (11%)	1 (2%)
Edema	1 (2%)		1 (2%)
Hemorrhage	3 (6%)	4 (22%)	1 (2%)
Spleen	(49)	(49)	(50)
Amyloid deposition			1 (2%)
Congestion	3 (6%)	1 (2%)	2 (4%)
Depletion lymphoid			1 (2%)
Fibrosis, focal	8 (16%)	5 (10%)	7 (14%)
Hematopoietic cell proliferation	2 (4%)	5 (10%)	5 (10%)
Hyperplasia, lymphoid	1 (2%)		
Infarct		1 (2%)	
Infiltration cellular, histiocyte	1 (2%)		1 (2%)
Inflammation, granulomatous, focal	1 (2%)		
Necrosis	2 (4%)	1 (2%)	
Pigmentation		1 (2%)	
Capsule, fibrosis, focal		1 (2%)	
Integumentary System			
Mammary gland	(48)	(18)	(50)
Dilatation	1 (2%)	2 (11%)	2 (4%)
Hyperplasia			1 (2%)
Skin	(49)	(21)	(50)
Cyst		1 (5%)	
Edema	1 (2%)	1 (5%)	
Hyperkeratosis			1 (2%)
Inflammation, acute	2 (4%)		
Inflammation, chronic		1 (5%)	
Epithelium, lip, hyperplasia			1 (2%)
Musculoskeletal System			
Bone	(50)	(14)	(50)
Hyperostosis	1 (2%)		
Osteomalacia	1 (2%)	1 (7%)	
Tarsal, inflammation, chronic active	1 (2%)		

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Nervous System			
Brain	(50)	(13)	(50)
Hemorrhage	1 (2%)		1 (2%)
Thrombus	2 (4%)		6 (12%)
Thrombus, multiple	1 (2%)		
Respiratory System			
Larynx	(26)		(27)
Inflammation, subacute	1 (4%)		
Ulcer	1 (4%)		
Lung	(50)	(30)	(50)
Congestion	1 (2%)	1 (3%)	
Fibrosis			1 (2%)
Foreign body			1 (2%)
Hemorrhage	2 (4%)	1 (3%)	1 (2%)
Hyperplasia, lymphoid			1 (2%)
Hyperplasia, adenomatous	4 (8%)	4 (13%)	2 (4%)
Infiltration cellular, histiocyte	4 (8%)	1 (3%)	1 (2%)
Pigmentation			1 (2%)
Interstitium, inflammation, chronic	1 (2%)	2 (7%)	5 (10%)
Nose	(49)	(14)	(50)
Inflammation, acute	1 (2%)		
Nasolacrimal duct, inflammation, acute	1 (2%)		2 (4%)
Sinus, foreign body		1 (7%)	2 (4%)
Sinus, fungus	4 (8%)	2 (14%)	3 (6%)
Sinus, inflammation, acute	8 (16%)	4 (29%)	9 (18%)
Turbinate, inflammation, chronic	5 (10%)		
Turbinate, inflammation, subacute	1 (2%)		
Turbinate, thrombus		1 (7%)	
Special Senses System			
Eye	(2)	(1)	(2)
Lens capsule, cataract	2 (100%)		
Retina, atrophy	2 (100%)		
Sclera, metaplasia, osseous			1 (50%)
Urinary System			
Kidney	(50)	(45)	(50)
Cyst	3 (6%)	2 (4%)	3 (6%)
Developmental malformation			1 (2%)
Fibrosis, focal		1 (2%)	1 (2%)
Hematopoietic cell proliferation	1 (2%)		
Nephropathy	48 (96%)	42 (93%)	46 (92%)
Pigmentation		1 (2%)	
Pelvis, dilatation			1 (2%)
Pelvis, mineralization	1 (2%)		3 (6%)
Renal tubule, degeneration	1 (2%)		1 (2%)
Urinary bladder	(50)	(14)	(49)
Hemorrhage	1 (2%)		

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF 4,4′-DIAMINO-2,2′-STILBENEDISULFONIC ACID, DISODIUM SALT

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TABLE B3 Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 116

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TABLE B5 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 121

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	12,500 ppm	25,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	5	3	1
Moribund kills	15	14	16
Survivors			
Terminal sacrifice	30	32	33
Died last week of study		1	
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, cecum	(49)	(8)	(50)
Intestine large, colon	(49)	(8)	(50)
Intestine large, rectum	(49)	(8)	(50)
Intestine small, ileum	(49)	(8)	(50)
Intestine small, jejunum	(49)	(8)	(50)
Liver	(50)	(42)	(50)
Neoplastic nodule	3 (6%)		1 (2%)
Mesentery	(2)	(2)	(3)
Pancreas	(49)	(8)	(50)
Salivary glands	(49)	(8)	(50)
Stomach, forestomach	(50)	(10)	(50)
Stomach, glandular	(49)	(10)	(50)
Leiomyosarcoma	1 (2%)		
Cardiovascular System			
Heart	(50)	(8)	(50)
Endocrine System			
Adrenal gland, cortex	(49)	(50)	(49)
Adenoma			2 (4%)
Adrenal gland, medulla	(49)	(50)	(49)
Pheochromocytoma malignant		2 (4%)	
Pheochromocytoma benign	2 (4%)	4 (8%)	5 (10%)
Islets, pancreatic	(49)	(8)	(50)
Adenoma			1 (2%)
Pituitary gland	(50)	(37)	(49)
Pars distalis, adenoma	15 (30%)	20 (54%)	13 (27%)
Pars distalis, adenoma, multiple			1 (2%)
Thyroid gland	(49)	(10)	(49)
C-cell, adenoma	2 (4%)		3 (6%)
C-cell, carcinoma	2 (4%)	2 (20%)	5 (10%)
Follicular cell, adenoma		1 (10%)	
General Body System			
None			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Genital System			
Clitoral gland	(47)	(13)	(46)
Adenoma	3 (6%)	5 (38%)	4 (9%)
Bilateral, adenoma	1 (2%)		
Ovary	(50)	(8)	(50)
Granulosa cell tumor benign			2 (4%)
Uterus	(50)	(22)	(50)
Leiomyosarcoma		1 (5%)	
Cervix, carcinoma	1 (2%)		
Cervix, leiomyosarcoma	1 (2%)		
Cervix, squamous cell carcinoma		1 (5%)	
Endometrium, polyp stromal	9 (18%)	8 (36%)	11 (22%)
Endometrium, sarcoma stromal		1 (5%)	
Hematopoietic System			
Bone marrow	(49)	(8)	(50)
Lymph node	(50)	(27)	(49)
Deep cervical, carcinoma, metastatic, thyroid gland	1 (2%)		
Lymph node, mesenteric	(50)	(8)	(49)
Axillary, mediastinal, adenocarcinoma, metastatic, skin	1 (2%)		
Spleen	(49)	(22)	(50)
Thymus	(48)	(8)	(49)
Thymoma benign	1 (2%)		1 (2%)
Integumentary System			
Mammary gland	(50)	(47)	(50)
Adenocarcinoma	1 (2%)	2 (4%)	3 (6%)
Adenoma	2 (4%)	1 (2%)	2 (4%)
Fibroadenoma	9 (18%)	17 (36%)	19 (38%)
Fibroadenoma, multiple	2 (4%)	4 (9%)	2 (4%)
Skin	(50)	(8)	(50)
Sebaceous gland, adenocarcinoma	1 (2%)		
Subcutaneous tissue, fibroma	1 (2%)		1 (2%)
Subcutaneous tissue, fibrosarcoma			2 (4%)
Subcutaneous tissue, lipoma			1 (2%)
Subcutaneous tissue, schwannoma benign	1 (2%)		
Musculoskeletal System			
None			
Nervous System			
Brain	(50)	(8)	(50)
Astrocytoma benign		1 (13%)	
Glioma benign	1 (2%)		1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Respiratory System			
Larynx	(33)		(38)
Lung	(50)	(29)	(50)
Adenocarcinoma, metastatic, skin	1 (2%)		
Alveolar/bronchiolar adenoma		1 (3%)	1 (2%)
Nose	(49)	(8)	(50)
Special Senses System			
Eye	(3)	(5)	(2)
Zymbal's gland	(2)	(1)	
Squamous cell carcinoma	2 (100%)		
Urinary System			
Kidney	(50)	(50)	(50)
Renal tubule, adenoma			1 (2%)
Renal tubule, carcinoma		1 (2%)	
Urinary bladder	(50)	(9)	(50)
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Leukemia mononuclear	16 (32%)	14 (28%)	15 (30%)
Tumor Summary			
Total animals with primary neoplasms[c]	45	44	50
Total primary neoplasms	77	86	97
Total animals with benign neoplasms	32	36	44
Total benign neoplasms	52	62	72
Total animals with malignant neoplasms	23	21	22
Total malignant neoplasms	25	24	25
Total animals with secondary neoplasms	2		
Total secondary neoplasms	3		

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except secondary tumors

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm

Number of Days on Study	099	528	559	578	578	603	638	644	653	664	664	682	684	686	686	687	700	713	713	728	729	729	729	732	732
Carcass ID Number	0161	0224	0245	0183	0205	0173	0191	0201	0172	0153	0242	0220	0213	0154	0225	0214	0118	0126	0223	0214	0181	0213	0224	0131	0144
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Mesentery																				+					
Pancreas	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma															X										
Tongue																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X			X		X		X		X	X					X					X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
C-cell, adenoma																		X							
C-cell, carcinoma																									
General Body System																									
None																									

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	732	732	732	732	732	732	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	
Carcass ID Number	0115	0165	0182	0192	0195	0204	0135	0141	0145	0152	0175	0184	0193	0194	0122	0122	0123	0155	0171	0211	0215	0231	0234	0241	0243	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule																X		X				X				3
Mesentery						+																				2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyosarcoma																										1
Tongue		+																	+							2
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign						X		X																		2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma			X		X	X								X		X	X						X			15
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma																				X						2
C-cell, carcinoma				X						X																2
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	0	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7
	9	2	5	7	7	0	3	4	5	6	6	8	8	8	8	8	0	1	1	2	2	2	2	3	3
	9	8	9	8	8	3	8	4	3	4	4	2	4	6	6	7	0	3	3	8	9	9	9	2	2
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	2	2	1	2	1	1	2	1	1	2	2	1	1	2	1	1	1	2	1	1	2	2	1	1
	6	2	4	8	0	7	9	0	7	5	4	0	3	5	2	4	8	6	2	4	8	1	4	3	4
	1	4	5	3	5	3	1	1	2	3	2	2	3	4	5	3	5	4	3	2	1	3	4	1	4
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Adenoma																									
Bilateral, adenoma																					X				
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cervix, carcinoma																									
Cervix, leiomyosarcoma			X																						
Endometrium, polyp stromal				X																	X				X
Vagina																								+	
Hematopoietic System																									
Blood																					+				
Bone marrow	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deep cervical, carcinoma, metastatic, thyroid gland																									
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Axillary, mediastinal, adenocarcinoma, metastatic, skin													X												
Spleen	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Adenoma																									
Fibroadenoma		X		X						X							X	X		X				X	
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sebaceous gland, adenocarcinoma													X												
Subcutaneous tissue, fibroma									X																
Subcutaneous tissue, schwannoma benign																									
Musculoskeletal System																									
Bone	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	732	732	732	732	732	732	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	015	016	018	019	019	020	013	014	014	015	017	018	019	019	012	022	023	015	017	011	015	013	034	014	023	Total Tissues/ Tumors
Genital System																										
Clitoral gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma						X									X	X										3
Bilateral, adenoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cervix, carcinoma	X																									1
Cervix, leiomyosarcoma																										1
Endometrium, polyp stromal		X				X		X									X		X	X						9
Vagina																										1
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Deep cervical, carcinoma, metastatic, thyroid gland										X																1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Axillary, mediastinal, adenocarcinoma, metastatic, skin																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymoma benign																				X						1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma					X																					1
Adenoma									X												X					2
Fibroadenoma			X																					X		9
Fibroadenoma, multiple						X													X							2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sebaceous gland, adenocarcinoma																										1
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, schwannoma benign																				X						1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	0	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7
	9	2	5	7	7	0	3	4	5	6	6	8	8	8	8	8	0	1	1	2	2	2	2	3	3
	9	8	9	8	8	3	8	4	3	4	4	2	4	6	6	7	0	3	3	8	9	9	9	2	2
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	2	2	1	2	1	1	2	1	1	2	2	1	1	2	1	1	1	2	1	1	2	2	1	1
	6	2	4	8	0	7	9	0	7	5	4	0	3	5	2	4	8	6	2	4	8	1	4	3	4
	1	4	5	3	5	3	1	1	2	3	2	2	3	4	5	3	5	4	3	2	1	3	4	1	4
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma benign																	X								
Respiratory System																									
Larynx																		+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, skin													X												
Nose	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																				+					+
Zymbal's gland						+	+																		
Squamous cell carcinoma						X	X																		
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X						X		X	X	X		X	X		X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	732	732	732	732	732	732	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	015	016	018	019	019	020	013	014	014	015	017	018	019	019	012	022	023	115	117	121	225	231	234	241	243	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma benign																										1
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	33
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																	+									3
Zymbal's gland																										2
Squamous cell carcinoma																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X			X		X				X				X		X				X					16

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	732	732	732	732	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	0473	0474	0482	0484	0382	0393	0395	0415	0442	0463	0475	0481	0483	0485	0374	0382	0384	0401	0402	0424	0435	0441	0453	0454	0461	Total Tissues/ Tumors
Alimentary System																										
Esophagus																										8
Intestine large																										8
Intestine large, cecum																										8
Intestine large, colon																										8
Intestine large, rectum																										8
Intestine small																										8
Intestine small, duodenum																										8
Intestine small, ileum																										8
Intestine small, jejunum																										8
Liver		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+	+		+	+	42
Mesentery		+																								2
Pancreas																										8
Salivary-glands																										8
Stomach																										10
Stomach, forestomach																										10
Stomach, glandular																										10
Cardiovascular System																										
Blood vessel																										1
Heart																										8
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																					X					2
Pheochromocytoma benign											X							X					X			4
Islets, pancreatic																										8
Parathyroid gland																										7
Pituitary gland		+		+	+	+	+	+		+	+		+	+	+	+	+	+		+		+	+	+	+	37
Pars distalis, adenoma		X		X			X			X				X	X	X				X			X	X	X	20
Thyroid gland								+				+														10
C-cell, carcinoma								X				X														2
Follicular cell, adenoma																										1
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	1	5	5	5	5	5	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7
	1	5	6	8	8	8	2	3	4	4	6	7	9	0	0	1	2	2	2	3	3	3	3	3	3
	8	8	5	3	3	8	1	0	3	3	3	1	8	6	8	8	2	9	9	2	2	2	2	2	2
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	3	4	4	4	4	4	4	3	4	4	4	4	3	4	3	4	3	4	4	4	4	4	4	4
	9	9	7	4	6	3	1	4	7	2	5	4	3	7	0	9	3	8	1	0	0	3	4	5	5
	1	4	1	5	4	1	4	4	2	1	5	2	3	1	3	2	2	5	1	4	5	4	3	1	2
Genital System																									
Clitoral gland	+	+	+	+	+	M	+	+			+			+			+								
Adenoma											X			X			X								
Ovary	+	+	+	+	+	+	+	+																	
Uterus	+	+	+	+	+	+	+	+	+				+	+			+			+	+				
Leiomyosarcoma							X																		
Cervix, squamous cell carcinoma																									
Endometrium, polyp stromal														X			X				X				
Endometrium, sarcoma stromal									X																
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+																	
Lymph node	+	+	+	+	+	+	+	+	+		+		+	+	+	+	+		+		+		+		+
Lymph node, mesenteric	+	+	+		+	+	+	+																	+
Spleen	+	+	+	+	+	+	+	+	+			+	+	+			+				+	+			
Thymus	+	+	+	+	+	+	+	+																	
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
Adenocarcinoma			X																						
Adenoma																									
Fibroadenoma						X			X		X			X			X				X				
Fibroadenoma, multiple				X														X		X					
Skin	+	+	+	+	+	+	+	+																	
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+																	
Nervous System																									
Brain	+	+	+	+	+	+	+	+																	
Astrocytoma benign		X																							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+							+		+			+		+			+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+																	
Trachea	+	+	+	+	+	+	+	+																	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	732	732	732	732	733	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	047 3	044 7	048 2	044 8	038 2	039 3	039 5	041 5	042 2	046 3	047 5	048 1	044 3	048 5	037 4	038 3	038 4	040 1	040 2	042 4	044 5	044 1	045 3	045 4	045 1	Total Tissues/ Tumors
Genital System																										
Clitoral gland			+					+	+																	13
Adenoma			X						X																	5
Ovary																										8
Uterus								+					+	+						+	+	+	+	+		22
Leiomyosarcoma																										1
Cervix, squamous cell carcinoma																					X					1
Endometrium, polyp stromal																				X	X	X	X	X		8
Endometrium, sarcoma stromal																										1
Hematopoietic System																										
Bone marrow																										8
Lymph node			+		+	+		+	+									+			+		+			27
Lymph node, mesenteric																										8
Spleen				+				+	+							+	+	+		+						22
Thymus																										8
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+	+	47
Adenocarcinoma											X															2
Adenoma										X																1
Fibroadenoma			X	X	X	X	X		X	X	X			X					X					X		17
Fibroadenoma, multiple																		X								4
Skin																										8
Musculoskeletal System																										
Bone																										8
Nervous System																										
Brain																										8
Astrocytoma benign																										1
Respiratory System																										
Lung	+	+	+			+	+		+	+	+				+			+	+		+	+	+	+	+	29
Alveolar/bronchiolar adenoma						X																				1
Nose																										8
Trachea																										8

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

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

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	732	732	732	732	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	0473	0474	0482	0484	0383	0393	0395	0415	0422	0463	0475	0481	0483	0485	0374	0383	0384	0401	0402	0424	0435	0441	0453	0454	0461	Total Tissues/ Tumors
Special Senses System																										
Eye												+														5
Zymbal's gland																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, carcinoma																										1
Urinary bladder																										9
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X				X		X								X	X					X		14

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm

Number of Days on Study	351	638	638	664	671	672	681	686	692	700	700	706	718	719	719	719	722	729	729	732	732	732	732	732	732
Carcass ID Number	0701	0651	0712	0694	0645	0674	0692	0663	0614	0633	0691	0642	0622	0634	0655	0684	0675	0624	0661	0621	0635	0654	0663	0703	0704
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																		X							
Mesentery																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx					+																				
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																	X								
Adrenal gland, medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign					X																			X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma								X		X									X				X		
Pars distalis, adenoma, multiple																									
Thyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									X
C-cell, carcinoma															X			X				X			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	732	732	732	732	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	0711	0713	0714	0723	0613	0623	0644	0652	0653	0695	0715	0721	0722	0724	0612	0625	0631	0662	0671	0673	0682	0685	0693	0705	0725	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule																										1
Mesentery							+	+										+								3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pharynx																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth							+																			1
Cardiovascular System																										
Blood vessel															+											1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma												X														2
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign				X					X							X										5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																X										1
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X										X		X	X	X		X			X		X			X	13
Pars distalis, adenoma, multiple		X																								1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma														X				X								3
C-cell, carcinoma																					X	X				5

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	351	638	638	664	671	672	681	686	692	700	700	706	718	719	719	719	722	729	729	732	732	732	732	732	732
Carcass ID Number	0701	0651	0712	0694	0645	0674	0669	0663	0641	0633	0691	0642	0622	0634	0655	0684	0675	0724	0661	0621	0635	0654	0663	0703	0704
General Body System																									
None																									
Genital System																									
Clitoral gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Adenoma					X								X												
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal	X				X				X							X		X			X				
Hematopoietic System																									
Blood								+										+							
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign																		X							
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma	X																		X				X		
Adenoma																									
Fibroadenoma		X	X	X				X	X				X		X			X		X					X
Fibroadenoma, multiple										X															
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma		X																							
Subcutaneous tissue, fibrosarcoma													X												
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	732	732	732	732	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	0711	0713	0714	0723	0613	0623	0644	0652	0653	0695	0715	0721	0722	0724	0612	0625	0631	0662	0671	0673	0682	0685	0693	0705	0725	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	46
Adenoma			X	X																						4
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor benign						X																	X			2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal								X							X				X			X	X			11
Hematopoietic System																										
Blood																										2
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymoma benign																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma																										3
Adenoma	X																							X		2
Fibroadenoma				X		X		X		X		X		X							X	X	X			19
Fibroadenoma, multiple					X																					2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, fibrosarcoma													X													2
Subcutaneous tissue, lipoma							X																			1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	351	638	638	664	671	672	681	688	692	700	700	706	718	719	719	719	722	729	729	732	732	732	732	732	732
Carcass ID Number	0701	0651	0712	0694	0645	0674	0692	0663	0641	0663	0691	0642	0622	0634	0655	0684	0675	0724	0661	0621	0635	0654	0663	0703	0704
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glioma benign																									
Respiratory System																									
Larynx												+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																		+							
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																					X				
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X				X	X		X		X	X	X	X	X	X							X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 25,000 ppm (continued)

Number of Days on Study	732	732	732	732	733	733	733	733	733	733	733	733	733	734	734	734	734	734	734	734	734	734	734	734	734	
Carcass ID Number	0711	0713	0714	0723	0613	0623	0644	0652	0653	0695	0715	0721	0722	0724	0612	0625	0631	0662	0671	0673	0682	0685	0693	0705	0725	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Glioma benign									X																	1
Respiratory System																										
Larynx	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	38
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																	X									1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+																									2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X							X								X				X			15

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm	12,500 ppm	25,000 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rates[a]	2/49 (4%)	4/50 (8%)	5/49 (10%)
Adjusted rates[b]	6.7%	12.1%	14.0%
Terminal rates[c]	2/30 (7%)	4/33 (12%)	4/33 (12%)
First incidence (days)	729 (T)	729 (T)	671
Life table tests[d]	P=0.204	P=0.380	P=0.261
Logistic regression tests[d]	P=0.219	P=0.380	P=0.265
Cochran-Armitage test[d]	P=0.168		
Fisher exact test[d]		P=0.349	P=0.218
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rates	2/49 (4%)	6/50 (12%)	5/49 (10%)
Adjusted rates	6.7%	17.4%	14.0%
Terminal rates	2/30 (7%)	5/33 (15%)	4/33 (12%)
First incidence (days)	729 (T)	706	671
Life table tests	P=0.232	P=0.167	P=0.261
Logistic regression tests	P=0.244	P=0.151	P=0.265
Cochran-Armitage test	P=0.186		
Fisher exact test		P=0.141	P=0.218
Clitoral Gland: Adenoma			
Overall rates	4/47 (9%)	5/13 (38%)[e]	4/46 (9%)
Adjusted rates	13.8%		11.1%
Terminal rates	4/29 (14%)		2/30 (7%)
First incidence (days)	729 (T)		671
Life table tests			P=0.603N
Logistic regression tests			P=0.587N
Fisher exact test			P=0.631
Liver: Neoplastic Nodule			
Overall rates	3/50 (6%)	0/42 (0%)	1/50 (2%)
Adjusted rates	10.0%	0.0%	3.0%
Terminal rates	3/30 (10%)	0/27 (0%)	1/33 (3%)
First incidence (days)	729 (T)	–[f]	729 (T)
Life table tests	P=0.160N	P=0.139N	P=0.271N
Logistic regression tests	P=0.160N	P=0.139N	P=0.271N
Cochran-Armitage test	P=0.182N		
Fisher exact test		P=0.156N	P=0.309N
Mammary Gland: Fibroadenoma			
Overall rates	11/50 (22%)	21/50 (42%)	21/50 (42%)
Adjusted rates	29.0%	53.1%	49.9%
Terminal rates	5/30 (17%)	15/33 (45%)	13/33 (39%)
First incidence (days)	528	583	638
Life table tests	P=0.074	P=0.061	P=0.082
Logistic regression tests	P=0.029	P=0.027	P=0.029
Cochran-Armitage test	P=0.023		
Fisher exact test		P=0.026	P=0.026

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Mammary Gland: Adenocarcinoma			
Overall rates	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rates	3.3%	5.1%	7.9%
Terminal rates	1/30 (3%)	1/33 (3%)	2/33 (6%)
First incidence (days)	729 (T)	565	351
Life table tests	P=0.246	P=0.524	P=0.334
Logistic regression tests	P=0.175	P=0.499	P=0.235
Cochran-Armitage test	P=0.222		
Fisher exact test		P=0.500	P=0.309
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	15/50 (30%)	20/37 (54%)	14/49 (29%)
Adjusted rates	38.1%	64.1%	39.4%
Terminal rates	8/30 (27%)	14/24 (58%)	12/33 (36%)
First incidence (days)	528	558	686
Life table tests	P=0.329N	P=0.107	P=0.380N
Logistic regression tests	P=0.446N	P=0.019	P=0.511N
Cochran-Armitage test	P=0.488N		
Fisher exact test		P=0.021	P=0.526N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rates	2.4%	0.0%	7.5%
Terminal rates	0/30 (0%)	0/33 (0%)	1/33 (3%)
First incidence (days)	653	–	638
Life table tests	P=0.209	P=0.510N	P=0.355
Logistic regression tests	P=0.168	P=0.501N	P=0.287
Cochran-Armitage test	P=0.176		
Fisher exact test		P=0.500N	P=0.309
Thyroid Gland (C-cell): Adenoma			
Overall rates	2/49 (4%)	0/10 (0%)[e]	3/49 (6%)
Adjusted rates	6.3%		9.1%
Terminal rates	1/30 (3%)		3/33 (9%)
First incidence (days)	713		729 (T)
Life table tests			P=0.547
Logistic regression tests			P=0.565
Fisher exact test			P=0.500
Thyroid Gland (C-cell): Carcinoma			
Overall rates	2/49 (4%)	2/10 (20%)[e]	5/49 (10%)
Adjusted rates	6.7%		14.5%
Terminal rates	2/30 (7%)		4/33 (12%)
First incidence (days)	729 (T)		719
Life table tests			P=0.262
Logistic regression tests			P=0.271
Fisher exact test			P=0.218

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rates	4/49 (8%)	2/10 (20%)[e]	8/49 (16%)
Adjusted rates	12.7%		23.3%
Terminal rates	3/30 (10%)		7/33 (21%)
First incidence (days)	713		719
Life table tests			P=0.233
Logistic regression tests			P=0.248
Fisher exact test			P=0.178
Uterus: Stromal Polyp			
Overall rates	9/50 (18%)	8/50 (16%)	11/50 (22%)
Adjusted rates	28.2%	22.7%	28.3%
Terminal rates	8/30 (27%)	6/33 (18%)	7/33 (21%)
First incidence (days)	578	706	351
Life table tests	P=0.444	P=0.419N	P=0.502
Logistic regression tests	P=0.379	P=0.479N	P=0.398
Cochran-Armitage test	P=0.350		
Fisher exact test		P=0.500N	P=0.402
Uterus: Stromal Polyp or Stromal Sarcoma			
Overall rates	9/50 (18%)	9/50 (18%)	11/50 (22%)
Adjusted rates	28.2%	24.6%	28.3%
Terminal rates	8/30 (27%)	6/33 (18%)	7/33 (21%)
First incidence (days)	578	643	351
Life table tests	P=0.448	P=0.525N	P=0.502
Logistic regression tests	P=0.375	P=0.591N	P=0.398
Cochran-Armitage test	P=0.352		
Fisher exact test		P=0.602N	P=0.402
All Organs: Mononuclear Cell Leukemia			
Overall rates	16/50 (32%)	14/50 (28%)	15/50 (30%)
Adjusted rates	43.9%	35.3%	34.1%
Terminal rates	10/30 (33%)	8/33 (24%)	5/33 (15%)
First incidence (days)	644	630	638
Life table tests	P=0.326N	P=0.337N	P=0.354N
Logistic regression tests	P=0.387N	P=0.399N	P=0.427N
Cochran-Armitage test	P=0.457N		
Fisher exact test		P=0.414N	P=0.500N
All Organs: Benign Tumors			
Overall rates	32/50 (64%)	36/50 (72%)	44/50 (88%)
Adjusted rates	77.3%	81.5%	97.7%
Terminal rates	21/30 (70%)	25/33 (76%)	32/33 (97%)
First incidence (days)	528	558	351
Life table tests	P=0.087	P=0.458	P=0.103
Logistic regression tests	P=0.007	P=0.263	P=0.008
Cochran-Armitage test	P=0.004		
Fisher exact test		P=0.260	P=0.005

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
All Organs: Malignant Tumors			
Overall rates	23/50 (46%)	21/50 (42%)	22/50 (44%)
Adjusted rates	55.3%	49.4%	49.2%
Terminal rates	12/30 (40%)	12/33 (36%)	11/33 (33%)
First incidence (days)	559	565	351
Life table tests	P=0.309N	P=0.341N	P=0.334N
Logistic regression tests	P=0.441N	P=0.414N	P=0.501N
Cochran-Armitage test	P=0.460N		
Fisher exact test		P=0.420N	P=0.500N
All Organs: Benign or Malignant Tumors			
Overall rates	45/50 (90%)	44/50 (88%)	50/50 (100%)
Adjusted rates	93.7%	91.6%	100.0%
Terminal rates	27/30 (90%)	29/33 (88%)	33/33 (100%)
First incidence (days)	528	558	351
Life table tests	P=0.522	P=0.318N	P=0.562N
Logistic regression tests	P=0.063	P=0.491N	P=0.051
Cochran-Armitage test	P=0.042		
Fisher exact test		P=0.500N	P=0.028

(T)Terminal sacrifice

[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.

[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.

[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus, statistical comparisons with the controls are not appropriate.

[f] Not applicable; no tumors in animal group

TABLE B4
Historical Incidence of Fibroadenomas of the Mammary Gland in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Overall Historical Incidence	
Total	314/800 (39.3%)
Standard deviation	15.1%
Range	8%–58%

[a] Data as of 29 March 1991

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	12,500 ppm	25,000 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	5	3	1
Moribund kills	15	14	16
Survivors			
Terminal sacrifice	30	32	33
Died last week of study		1	
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, cecum	(49)	(8)	(50)
Parasite metazoan	1 (2%)		
Intestine large, colon	(49)	(8)	(50)
Mineralization		1 (13%)	
Parasite metazoan	4 (8%)		
Intestine large, rectum	(49)	(8)	(50)
Mineralization		1 (13%)	
Parasite metazoan	1 (2%)		
Liver	(50)	(42)	(50)
Angiectasis	1 (2%)	1 (2%)	7 (14%)
Congestion	2 (4%)	3 (7%)	
Cytologic alterations	1 (2%)	3 (7%)	3 (6%)
Cytologic alterations, multiple	23 (46%)	12 (29%)	22 (44%)
Fibrosis, focal		1 (2%)	
Hematopoietic cell proliferation			1 (2%)
Hemorrhage	1 (2%)		1 (2%)
Hepatodiaphragmatic nodule	16 (32%)	10 (24%)	8 (16%)
Hyperplasia, focal	5 (10%)	4 (10%)	4 (8%)
Hyperplasia, multifocal		2 (5%)	
Inflammation, chronic	13 (26%)	12 (29%)	13 (26%)
Mitotic alteration	1 (2%)		
Necrosis, multifocal	1 (2%)		
Pigmentation			1 (2%)
Thrombus			1 (2%)
Vacuolization cytoplasmic		1 (2%)	1 (2%)
Bile duct, hyperplasia	19 (38%)	14 (33%)	16 (32%)
Centrilobular, necrosis	1 (2%)	2 (5%)	1 (2%)
Centrilobular, vacuolization cytoplasmic	5 (10%)	1 (2%)	4 (8%)
Mesentery	(2)	(2)	(3)
Hyperplasia, lymphoid			1 (33%)
Fat, necrosis	2 (100%)	2 (100%)	2 (67%)
Pancreas	(49)	(8)	(50)
Inflammation, chronic			1 (2%)
Acinus, atrophy, diffuse	1 (2%)		
Acinus, atrophy, focal	13 (27%)	1 (13%)	23 (46%)
Acinus, hyperplasia, focal			1 (2%)
Artery, inflammation, chronic			2 (4%)
Artery, inflammation, subacute			1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Alimentary System (continued)			
Pharynx			(1)
Palate, inflammation, acute			1 (100%)
Salivary glands	(49)	(8)	(50)
Atrophy, focal	1 (2%)		
Cyst			1 (2%)
Stomach, forestomach	(50)	(10)	(50)
Inflammation, acute	2 (4%)		1 (2%)
Inflammation, chronic		1 (10%)	
Ulcer		1 (10%)	4 (8%)
Epithelium, hyperplasia	2 (4%)		1 (2%)
Stomach, glandular	(49)	(10)	(50)
Edema	1 (2%)		
Erosion	1 (2%)	2 (20%)	1 (2%)
Inflammation, acute	1 (2%)		1 (2%)
Mineralization	1 (2%)	1 (10%)	
Pigmentation	2 (4%)		
Ulcer			2 (4%)
Tongue	(2)		
Epithelium, hyperplasia	2 (100%)		
Tooth			(1)
Inflammation, chronic active			1 (100%)
Cardiovascular System			
Blood vessel		(1)	(1)
Aorta, mineralization		1 (100%)	
Artery, inflammation, chronic			1 (100%)
Heart	(50)	(8)	(50)
Cardiomyopathy	25 (50%)	4 (50%)	28 (56%)
Mineralization	1 (2%)	1 (13%)	
Atrium, thrombus	2 (4%)		4 (8%)
Myocardium, necrosis			1 (2%)
Endocrine System			
Adrenal gland, cortex	(49)	(50)	(49)
Amyloid deposition		1 (2%)	1 (2%)
Congestion	2 (4%)		
Hemorrhage		1 (2%)	
Hyperplasia, focal			1 (2%)
Hypertrophy, focal		1 (2%)	
Necrosis, focal	2 (4%)	2 (4%)	1 (2%)
Vacuolization cytoplasmic, focal	6 (12%)	8 (16%)	9 (18%)
Adrenal gland, medulla	(49)	(50)	(49)
Hyperplasia	9 (18%)	5 (10%)	7 (14%)
Infiltration cellular, lymphocyte	1 (2%)		

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Endocrine System (continued)			
Islets, pancreatic	(49)	(8)	(50)
Hyperplasia			2 (4%)
Parathyroid gland	(47)	(7)	(48)
Hyperplasia		1 (14%)	
Pituitary gland	(50)	(37)	(49)
Hemorrhage	1 (2%)		
Pars distalis, angiectasis	15 (30%)	9 (24%)	15 (31%)
Pars distalis, cyst	7 (14%)	2 (5%)	3 (6%)
Pars distalis, cyst, multiple			1 (2%)
Pars distalis, hyperplasia	9 (18%)	6 (16%)	9 (18%)
Pars distalis, infarct			1 (2%)
Pars distalis, pigmentation		1 (3%)	
Pars nervosa, cyst	1 (2%)		
Pars nervosa, ectopic tissue		1 (3%)	1 (2%)
Thyroid gland	(49)	(10)	(49)
Cyst	1 (2%)		
C-cell, hyperplasia	8 (16%)		5 (10%)
General Body System			
None			
Genital System			
Clitoral gland	(47)	(13)	(46)
Dilatation	1 (2%)		
Hyperplasia			1 (2%)
Inflammation, acute	6 (13%)	1 (8%)	2 (4%)
Ovary	(50)	(8)	(50)
Cyst	10 (20%)		3 (6%)
Inflammation, chronic	1 (2%)		
Mineralization		1 (13%)	
Uterus	(50)	(22)	(50)
Angiectasis			1 (2%)
Fibrosis			1 (2%)
Hemorrhage	1 (2%)		
Inflammation, chronic	1 (2%)		
Cervix, inflammation, acute		1 (5%)	
Endometrium, hyperplasia, cystic	5 (10%)	3 (14%)	4 (8%)
Vagina	(1)		
Inflammation, acute	1 (100%)		
Hematopoietic System			
Bone marrow	(49)	(8)	(50)
Fibrosis	1 (2%)		1 (2%)
Hyperplasia		2 (25%)	1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Hematopoietic System (continued)			
Lymph node	(50)	(27)	(49)
Axillary, hemorrhage			1 (2%)
Axillary, hyperplasia, lymphoid			2 (4%)
Bronchial, hemorrhage	1 (2%)		
Iliac, cyst	1 (2%)	1 (4%)	
Iliac, hemorrhage		1 (4%)	
Iliac, hyperplasia, lymphoid		2 (7%)	
Inguinal, hyperplasia, lymphoid		1 (4%)	
Lymphocyte, mandibular, necrosis			1 (2%)
Mandibular, cyst	4 (8%)		1 (2%)
Mandibular, hemorrhage	5 (10%)		4 (8%)
Mediastinal, hemorrhage	7 (14%)	7 (26%)	14 (29%)
Mediastinal, pigmentation	1 (2%)		1 (2%)
Mediastinal, lymphocyte, necrosis			1 (2%)
Prefemoral, hyperplasia, lymphoid		1 (4%)	
Lymph node, mesenteric	(50)	(8)	(49)
Hemorrhage	4 (8%)	1 (13%)	4 (8%)
Hyperplasia, lymphoid	1 (2%)		
Spleen	(49)	(22)	(50)
Congestion	1 (2%)		
Fibrosis, focal	1 (2%)	1 (5%)	2 (4%)
Hematopoietic cell proliferation	2 (4%)	3 (14%)	2 (4%)
Hemorrhage		1 (5%)	
Inflammation, granulomatous, focal			1 (2%)
Necrosis, focal	1 (2%)	1 (5%)	
Pigmentation	2 (4%)	2 (9%)	1 (2%)
Thymus	(48)	(8)	(49)
Cyst	1 (2%)		1 (2%)
Integumentary System			
Mammary gland	(50)	(47)	(50)
Dilatation	6 (12%)	4 (9%)	1 (2%)
Hyperplasia	4 (8%)	1 (2%)	3 (6%)
Skin	(50)	(8)	(50)
Cyst	1 (2%)		
Subcutaneous tissue, abscess	1 (2%)		1 (2%)
Subcutaneous tissue, edema	1 (2%)		
Subcutaneous tissue, thrombus, multiple	1 (2%)		
Musculoskeletal System			
Bone	(49)	(8)	(50)
Osteoporosis		1 (13%)	
Nervous System			
Brain	(50)	(8)	(50)
Thrombus	1 (2%)		

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	12,500 ppm	25,000 ppm
Respiratory System			
Larynx	(33)		(38)
Inflammation, chronic	1 (3%)		
Lung	(50)	(29)	(50)
Foreign body	1 (2%)		
Hemorrhage	12 (24%)	13 (45%)	4 (8%)
Hyperplasia, adenomatous	2 (4%)	3 (10%)	1 (2%)
Infiltration cellular, histiocyte	7 (14%)	2 (7%)	1 (2%)
Thrombus	1 (2%)		
Interstitium, inflammation, chronic	3 (6%)		10 (20%)
Nose	(49)	(8)	(50)
Metaplasia, squamous		1 (13%)	
Nasolacrimal duct, fungus	1 (2%)		
Nasolacrimal duct, inflammation, acute	1 (2%)	1 (13%)	1 (2%)
Sinus, foreign body	1 (2%)		
Sinus, fungus	1 (2%)	1 (13%)	1 (2%)
Sinus, inflammation, acute	3 (6%)	2 (25%)	1 (2%)
Turbinate, inflammation, chronic			1 (2%)
Special Senses System			
Eye	(3)	(5)	(2)
Cornea, inflammation, chronic		1 (20%)	
Iris, inflammation, chronic	1 (33%)		
Lens capsule, cataract	1 (33%)	5 (100%)	2 (100%)
Retina, atrophy	1 (33%)	5 (100%)	2 (100%)
Sclera, metaplasia, osseous	1 (33%)	1 (20%)	
Urinary System			
Kidney	(50)	(50)	(50)
Cyst	1 (2%)		
Fibrosis, focal		1 (2%)	
Inflammation, acute			3 (6%)
Nephropathy	46 (92%)	47 (94%)	47 (94%)
Pigmentation	1 (2%)	3 (6%)	3 (6%)
Artery, inflammation, chronic		1 (2%)	
Pelvis, dilatation	1 (2%)		
Pelvis, inflammation, acute	1 (2%)	1 (2%)	
Pelvis, inflammation, subacute	1 (2%)		
Pelvis, mineralization	8 (16%)	30 (60%)	37 (74%)
Pelvis, epithelium, hyperplasia		1 (2%)	
Urinary bladder	(50)	(9)	(50)
Hemorrhage		1 (11%)	
Inflammation, acute	1 (2%)		
Inflammation, chronic	1 (2%)		

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF 4,4′-DIAMINO-2,2′-STILBENEDISULFONIC ACID, DISODIUM SALT

TABLE C1 Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 129

TABLE C2 Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 132

TABLE C3 Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 150

TABLE C4 Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 153

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	6,250 ppm	12,500 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	4	6	2
Moribund kills	3	3	6
Survivors			
Terminal sacrifice	43	40	42
Missing		1	
Animals examined microscopically	50	49	50
Alimentary System			
Intestine large, cecum	(48)	(4)	(48)
Intestine small, duodenum	(47)	(4)	(46)
Intestine small, ileum	(48)	(5)	(48)
Intestine small, jejunum	(47)	(14)	(48)
Liver	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	
Hepatocellular carcinoma	2 (4%)	3 (6%)	3 (6%)
Hepatocellular carcinoma, multiple	1 (2%)		
Hepatocellular adenoma	2 (4%)	9 (18%)	5 (10%)
Hepatocellular adenoma, multiple			1 (2%)
Hepatocholangiocarcinoma	1 (2%)		
Pancreas	(49)	(7)	(50)
Hemangioma	1 (2%)		
Stomach, forestomach	(49)	(6)	(50)
Stomach, glandular	(49)	(6)	(50)
Cardiovascular System			
Heart	(50)	(8)	(50)
Endocrine System			
Adrenal gland	(50)	(7)	(50)
Capsule, adenoma			1 (2%)
Adrenal gland, cortex	(50)	(7)	(50)
Adrenal gland, medulla	(50)	(6)	(48)
Pheochromocytoma benign			2 (4%)
Thyroid gland	(50)	(7)	(49)
Follicular cell, adenoma	1 (2%)		
General Body System			
None			
Genital System			
Epididymis	(50)	(9)	(50)
Prostate	(50)	(7)	(48)
Seminal vesicle	(49)	(9)	(50)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Hematopoietic System			
Bone marrow	(50)	(8)	(50)
Lymph node	(48)	(13)	(47)
Sarcoma, metastatic, skin		1 (8%)	
Lymph node, mesenteric	(45)	(10)	(45)
Spleen	(50)	(39)	(50)
Hemangiosarcoma		1 (3%)	
Hemangiosarcoma, metastatic, liver		1 (3%)	
Sarcoma	1 (2%)		
Thymus	(44)	(4)	(45)
Integumentary System			
Skin	(50)	(20)	(50)
Carcinoma		2 (10%)	
Subcutaneous tissue, fibroma	2 (4%)	1 (5%)	1 (2%)
Subcutaneous tissue, fibrosarcoma	2 (4%)	1 (5%)	2 (4%)
Subcutaneous tissue, hemangioma			1 (2%)
Subcutaneous tissue, sarcoma	1 (2%)	1 (5%)	2 (4%)
Subcutaneous tissue, schwannoma NOS			1 (2%)
Sweat gland, adenocarcinoma	1 (2%)		
Musculoskeletal System			
Skeletal muscle	(1)	(2)	(1)
Fibrosarcoma, metastatic		1 (50%)	
Nervous System			
Brain	(50)	(40)	(49)
Respiratory System			
Lung	(50)	(48)	(50)
Alveolar/bronchiolar adenoma	10 (20%)	3 (6%)	7 (14%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)		
Alveolar/bronchiolar carcinoma	3 (6%)	1 (2%)	4 (8%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	1 (2%)	
Nose	(50)	(8)	(49)
Special Senses System			
Harderian gland	(1)	(1)	
Adenoma	1 (100%)	1 (100%)	
Urinary System			
Kidney	(50)	(14)	(50)
Urinary bladder	(48)	(7)	(49)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Systemic Lesions			
Multiple organs[b]	(50)	(49)	(50)
Lymphoma malignant histiocytic		1 (2%)	1 (2%)
Lymphoma malignant lymphocytic	1 (2%)	3 (6%)	1 (2%)
Lymphoma malignant mixed		2 (4%)	2 (4%)
Lymphoma malignant undifferentiated cell	1 (2%)		2 (4%)
Tumor Summary			
Total animals with primary neoplasms[c]	23	24	25
Total primary neoplasms	34	30	36
Total animals with benign neoplasms	15	10	15
Total benign neoplasms	19	14	18
Total animals with malignant neoplasms	11	15	16
Total malignant neoplasms	15	16	17
Total animals with secondary neoplasms	1	4	
Total secondary neoplasms	1	4	
Total animals with neoplasms uncertain-benign or malignant			1
Total uncertain neoplasms			1

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with tissue examined microscopically
[c] Primary tumors: all tumors except secondary tumors

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm

Number of Days on Study	0	0	0	1	3	3	5	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	4	5	7	3	2	3	6	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3
	5	3	0	1	1	7	8	9	9	9	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0	0
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	0	0	1	7	3	6	8	1	1	2	2	4	6	8	8	9	9	0	2	3	4	4	4	6	7	
	1	2	1	1	2	4	4	1	3	2	5	2	3	1	5	1	3	5	3	5	1	3	5	5	3	
Alimentary System																										
Esophagus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	M	+	+	+	+	+	+	
Gallbladder	+	+	A	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	
Intestine large	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, cecum	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, colon	M	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, rectum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, duodenum	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, ileum	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, jejunum	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hemangiosarcoma																										
Hepatocellular carcinoma										X						X										
Hepatocellular carcinoma, multiple																					X					
Hepatocellular adenoma															X											
Hepatocholangiocarcinoma																										
Mesentery																										
Pancreas	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hemangioma																										
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach, forestomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach, glandular	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Islets, pancreatic	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Parathyroid gland	M	M	+	M	+	+	+	+	+	M	+	+	+	M	+	+	+	M	M	+	+	+	+	+	+	
Pituitary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Follicular cell, adenoma																										

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

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0092	0095	0013	0125	0024	0033	0044	0054	0061	0072	0074	0075	0083	0104	0122	0125	0021	0034	0051	0052	0055	0062	0112	0121	0123	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	45
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	46
Intestine large, rectum	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																							X			1
Hepatocellular carcinoma																										2
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma												X														2
Hepatocholangiocarcinoma				X																						1
Mesentery				+																						1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangioma																						X				1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	M	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	38
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma	X																									1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	045	053	070	131	321	337	568	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730
Carcass ID Number	0101	0112	0101	0071	0032	0064	0084	0011	0013	0022	0025	0042	0063	0081	0085	0091	0093	0105	0023	0035	0041	0043	0045	0065	0073
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis	+	+		+																						
Preputial gland				+				+													+					
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																					X					
Thymus	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																			X							
Subcutaneous tissue, fibrosarcoma																					X					
Subcutaneous tissue, sarcoma												X														
Sweat gland, adenocarcinoma													X													
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle												+														
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0092	0095	0013	0125	0224	0323	0444	0545	0061	0072	0074	0075	0083	0104	0122	0125	0121	0124	0151	0152	0155	0062	0122	0121	0123	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										3
Preputial gland							+												+	+						6
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	45
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma																										1
Thymus	M	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	44
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																	X									2
Subcutaneous tissue, fibrosarcoma							X																			2
Subcutaneous tissue, sarcoma																										1
Sweat gland, adenocarcinoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	0	0	0	1	3	3	5	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	4	5	7	3	2	3	6	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3
	5	3	0	1	1	7	8	9	9	9	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	0	0	1	7	3	6	8	1	1	2	2	4	6	8	8	9	9	0	2	3	4	4	4	6	7
	1	2	1	1	2	4	4	1	3	2	5	2	3	1	5	1	3	5	3	5	1	3	5	5	3
Respiratory System																									
Larynx								+		+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma										X					X	X									
Alveolar/bronchiolar adenoma, multiple																						X			
Alveolar/bronchiolar carcinoma																									X
Hepatocellular carcinoma, metastatic, liver																					X				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye									+																
Harderian gland																									
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra		+																							
Urinary bladder	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic																									
Lymphoma malignant undifferentiated cell type																X									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732		
Carcass ID Number	0092	0095	0013	0125	0024	0033	0044	0054	0061	0072	0074	0075	0083	0104	0122	0125	0021	0023	0051	0052	0055	0062	0112	0121	0123	Total Tissues/ Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X			X							X						X					X		X	X	10
Alveolar/bronchiolar adenoma, multiple																					X					2
Alveolar/bronchiolar carcinoma							X		X																	3
Hepatocellular carcinoma, metastatic, liver																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																							+			1
Eye																										1
Harderian gland						+																				1
Adenoma						X																				1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra																	+									2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic					X																					1
Lymphoma malignant undifferentiated cell type																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm

Number of Days on Study	209	279	439	605	607	628	696	711	714	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730
Carcass ID Number	351	312	163	343	282	254	314	227	229	225	236	265	285	302	311	313	343	353	354	251	271	284	304	305
Alimentary System																									
Esophagus	+	+	A	+	+	+	+	+																	
Gallbladder	A	A	A	A	+	+	+	+																	
Intestine large	+	+	+	A	+	+	+	+																	
Intestine large, cecum	A	A	A	A	+	+	+	+																	
Intestine large, colon	A	+	A	A	+	+	+	+																	
Intestine large, rectum	+	+	+	A	+	+	+	+																	
Intestine small	A	+	A	A	+	+	+	+													+		+		
Intestine small, duodenum	A	A	A	A	+	+	+	+																	
Intestine small, ileum	A	A	A	A	+	+	+	+															+		
Intestine small, jejunum	A	A	A	A	+	+	+	+													+				
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hemangiosarcoma								X																	
Hepatocellular carcinoma																									
Hepatocellular adenoma										X	X	X				X				X					
Pancreas	+	+	A	+	+	+	+	+																	
Salivary glands	+	+	A	+	+	+	+	+																	
Stomach	A	+	A	+	+	+	+	+																	
Stomach, forestomach	A	+	A	+	+	+	+	+																	
Stomach, glandular	A	+	A	+	+	+	+	+																	
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+																	
Endocrine System																									
Adrenal gland	+	+	A	+	+	+	+	+																	
Adrenal gland, cortex	+	+	A	+	+	+	+	+																	
Adrenal gland, medulla	+	+	A	+	+	M	+	+																	
Islets, pancreatic	+	+	A	+	+	+	+	+																	
Parathyroid gland	M	+	A	+	M	M	+	+																	
Pituitary gland	+	+	+	+	+	M	+	+																	
Thyroid gland	+	+	A	+	+	+	+	+																	
General Body System																									
None																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm (continued)

Number of Days on Study	730	730	730	730	730	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	031	032	032	033	034	035	036	037	039	039	032	034	034	034	035	035	037	030	031	033	033	035	036	036	036	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus																										7
Gallbladder																										4
Intestine large																										7
Intestine large, cecum																										4
Intestine large, colon																										5
Intestine large, rectum																										7
Intestine small					+		+			+			+	+		+	+	+							+	16
Intestine small, duodenum																										4
Intestine small, ileum																										5
Intestine small, jejunum					+		+			+			+	+		+	+	+							+	14
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma																										1
Hepatocellular carcinoma		X						X			X															3
Hepatocellular adenoma								X				X			X							X				9
Pancreas																										7
Salivary glands																										7
Stomach																										6
Stomach, forestomach																										6
Stomach, glandular																										6
Cardiovascular System																										
Heart																										8
Endocrine System																										
Adrenal gland																										7
Adrenal gland, cortex																										7
Adrenal gland, medulla																										6
Islets, pancreatic																										7
Parathyroid gland																										4
Pituitary gland																										7
Thyroid gland																										7
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm (continued)

Number of Days on Study	2	2	4	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	0	7	3	0	0	2	9	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3
	9	9	9	5	7	8	6	1	4	9	9	9	9	9	9	9	9	9	9	9	0	0	0	0	0
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	3	3	3	2	2	3	2	2	2	2	2	2	2	3	3	3	3	3	3	2	2	2	3	3
	5	1	6	3	8	5	1	7	9	5	6	6	8	5	0	1	3	4	5	5	5	7	8	0	0
	1	2	1	4	1	4	4	4	1	2	3	5	5	2	1	1	1	3	3	4	1	1	4	4	5
Genital System																									
Epididymis	+	+	+	+	+	+	+	+									+								
Penis		+																							
Preputial gland										+															+
Prostate	+	A	+	+	+	+	+	+																	
Seminal vesicle	+	+	+		+	M	+	+																	
Testes	+	+	+	+	+	+	+	+																	
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+																	
Lymph node	+	+	A	+	+	+	+	+																	
Sarcoma, metastatic, skin				X																					
Lymph node, mesenteric	+	+	A	A	+	+	+	+																	
Spleen	+	+	A	+	+	+	+	+	+					+	+	+	+	+	+	+	+		+		
Hemangiosarcoma																		X							
Hemangiosarcoma, metastatic, liver								X																	
Thymus	+	M	M	M	M	+	+	+																	
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	+																	
Skin	+	+	+	+	+	+	+	+			+	+	+	+								+	+	+	+
Carcinoma																								X	
Subcutaneous tissue, fibroma											X														
Subcutaneous tissue, fibrosarcoma					X																				
Subcutaneous tissue, sarcoma				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+									+								
Skeletal muscle				+	+																				
Fibrosarcoma, metastatic					X																				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+		+		+	+		+	+		+	+	+	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm (continued)

Number of Days on Study	730	730	730	730	730	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0313	0324	0325	0333	0341	0253	0262	0273	0292	0293	0321	0342	0344	0345	0352	0355	0372	0301	0315	0332	0335	0355	0362	0363	0365	Total Tissues/ Tumors
Genital System																										
Epididymis																										9
Penis																										1
Preputial gland									+						+	+				+			+			7
Prostate																										7
Seminal vesicle								+									+					+				9
Testes																	+									9
Hematopoietic System																										
Bone marrow																										8
Lymph node	+															+		+		+			+		+	13
Sarcoma, metastatic, skin																										1
Lymph node, mesenteric	+																			+			+		+	10
Spleen	+		+		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	39
Hemangiosarcoma																										1
Hemangiosarcoma, metastatic, liver																										1
Thymus																										4
Integumentary System																										
Mammary gland																										1
Skin	+						+									+			+							20
Carcinoma							X																			2
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone																										9
Skeletal muscle																										2
Fibrosarcoma, metastatic																										1
Nervous System																										
Brain	+		+	+	+	+		+		+	+		+	+	+	+	+	+	+	+	+	+	+		+	40

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm (continued)

Number of Days on Study	209	279	439	605	607	628	696	711	711	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730
Carcass ID Number	0351	0312	0361	0334	0281	0225	0331	0227	0229	0225	0226	0226	0228	0230	0231	0233	0234	0235	0235	0234	0225	0227	0228	0230	0230
Respiratory System																									
Lung	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma												X					X			X					
Alveolar/bronchiolar carcinoma																									
Hepatocellular carcinoma, metastatic, liver																									
Nose	+	+	+	+	+	+	+	+																	
Trachea	+	+	A	+	+	+	+	+																	
Special Senses System																									
Eye																+									
Harderian gland																+									
Adenoma																X									
Urinary System																									
Kidney	+	+	A	+	+	+	+	+							+	+		+							
Urinary bladder	+	A	+	+	+	+	+	+																	
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																									
Lymphoma malignant lymphocytic							X																	X	
Lymphoma malignant mixed						X			X																

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm (continued)

Number of Days on Study	730	730	730	730	730	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732		
Carcass ID Number	0313	0324	0325	0333	0341	0253	0262	0273	0292	0293	0321	0342	0344	0345	0352	0255	0272	0301	0315	0323	0335	0355	0362	0363	0365	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma																										3
Alveolar/bronchiolar carcinoma																	X									1
Hepatocellular carcinoma, metastatic, liver		X																								1
Nose																										8
Trachea																										7
Special Senses System																										
Eye																										1
Harderian gland																										1
Adenoma																										1
Urinary System																										
Kidney									+		+						+						+			14
Urinary bladder																										7
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant histiocytic																X										1
Lymphoma malignant lymphocytic																									X	3
Lymphoma malignant mixed																										2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm

Number of Days on Study	022	022	065	130	133	438	576	645	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730
Carcass ID Number	051	051	060	053	053	054	060	051	049	049	050	051	051	052	053	055	057	058	059	049	049	045	052	054	054
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	M	+	M	A	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+
Intestine small, ileum	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																		X	X	X					
Hepatocellular adenoma											X										X				
Hepatocellular adenoma, multiple																		X							
Mesentery																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Tooth									+	+															
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Capsule, adenoma																					X				
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	+	+	+	+	+	+	M	M	+	+	M	M	M	+	+	M	M	+	M	M	+	+	M
Pituitary gland	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Thyroid gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	730	730	730	730	730	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0554	0555	0565	0591	0592	0502	0522	0523	0551	0562	0572	0584	0594	0595	0603	0505	0533	0534	0563	0564	0571	0575	0583	0585	0602	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	44
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																										3
Hepatocellular adenoma		X															X							X		5
Hepatocellular adenoma, multiple																										1
Mesentery																						+				1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue												+														1
Tooth																			+							3
Cardiovascular System																										
Blood vessel														+												1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma																										1
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma benign						X																			X	2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	M	+	M	M	+	+	+	M	+	+	M	+	+	M	+	+	+	M	+	+	M	+	M	M	M	27
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	44
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	022	022	065	130	133	438	576	645	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730
Carcass ID Number	051	051	060	153	153	154	160	154	149	149	150	153	151	154	153	157	158	159	149	149	152	154	144	145
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis	+		+	+	+																				
Preputial gland				+	+							+			+				+				+		
Prostate	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																									
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma									X																
Subcutaneous tissue, fibrosarcoma																							X	X	
Subcutaneous tissue, hemangioma																									
Subcutaneous tissue, sarcoma											X														X
Subcutaneous tissue, schwannoma NOS																				X					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle											+														
Nervous System																									
Brain	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0554	0555	0566	0591	0592	0502	0522	0523	0551	0562	0572	0584	0594	0595	0603	0505	0533	0534	0563	0564	0571	0575	0583	0585	0602	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										4
Preputial gland				+										+		+	+							+		11
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	45
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	45
Integumentary System																										
Mammary gland	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, fibrosarcoma																										2
Subcutaneous tissue, hemangioma													X													1
Subcutaneous tissue, sarcoma																										2
Subcutaneous tissue, schwannoma NOS																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	0	0	0	1	1	4	5	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	2	2	6	3	3	3	7	4	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3
	2	2	5	0	1	8	6	5	9	9	9	9	9	9	9	9	9	9	9	0	0	0	0	0	0
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	5	6	5	5	5	6	5	4	4	5	5	5	5	5	5	5	5	5	4	4	5	5	5	5
	1	1	0	3	3	4	0	1	9	9	0	1	2	2	3	5	7	8	9	9	9	2	4	4	4
	1	2	1	1	2	3	4	4	3	4	1	3	1	4	5	3	3	1	3	1	5	5	1	4	5
Respiratory System																									
Larynx									+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																					X			X	
Alveolar/bronchiolar carcinoma																			X						
Nose	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
None																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ureter																									
Urethra			+		+																				
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic						X																			
Lymphoma malignant lymphocytic																									
Lymphoma malignant mixed															X										
Lymphoma malignant undifferentiated cell type								X														X			

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	730	730	730	730	730	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0554	0555	0565	0591	0592	0552	0522	0553	0551	0562	0572	0584	0594	0595	0603	0505	0533	0534	0563	0564	0571	0575	0583	0585	0602	Total Tissues/ Tumors
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	39
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X				X					X			X	X												7
Alveolar/bronchiolar carcinoma								X						X			X									4
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Ureter			+																							1
Urethra																										2
Urinary bladder	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic					X																					1
Lymphoma malignant mixed			X																							2
Lymphoma malignant undifferentiated cell type																										2

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm	6,250 ppm	12,500 ppm
Liver: Hepatocellular Adenoma			
Overall rates[a]	2/50 (4%)	9/49 (18%)	6/50 (12%)
Adjusted rates[b]	4.7%	22.5%	14.3%
Terminal rates[c]	2/43 (5%)	9/40 (23%)	6/42 (14%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests[d]	P=0.126	P=0.020	P=0.127
Logistic regression tests[d]	P=0.126	P=0.020	P=0.127
Cochran-Armitage test[d]	P=0.135		
Fisher exact test[d]		P=0.023	P=0.134
Liver: Hepatocellular Carcinoma			
Overall rates	3/50 (6%)	3/49 (6%)	3/50 (6%)
Adjusted rates	7.0%	7.5%	7.1%
Terminal rates	3/43 (7%)	3/40 (8%)	3/42 (7%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests	P=0.571	P=0.629	P=0.652
Logistic regression tests	P=0.571	P=0.629	P=0.652
Cochran-Armitage test	P=0.583N		
Fisher exact test		P=0.651	P=0.661N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates	5/50 (10%)	11/49 (22%)	8/50 (16%)
Adjusted rates	11.6%	27.5%	19.0%
Terminal rates	5/43 (12%)	11/40 (28%)	8/42 (19%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests	P=0.230	P=0.061	P=0.259
Logistic regression tests	P=0.230	P=0.061	P=0.259
Cochran-Armitage test	P=0.248		
Fisher exact test		P=0.079	P=0.277
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	12/50 (24%)	3/48 (6%)	7/50 (14%)
Adjusted rates	27.9%	7.5%	16.7%
Terminal rates	12/43 (28%)	3/40 (8%)	7/42 (17%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests	P=0.110N	P=0.017N	P=0.164N
Logistic regression tests	P=0.110N	P=0.017N	P=0.164N
Cochran-Armitage test	P=0.103N		
Fisher exact test		P=0.014N	P=0.154N
Lung: Alveolar/bronchiolar Carcinoma			
Overall rates	3/50 (6%)	1/48 (2%)	4/50 (8%)
Adjusted rates	7.0%	2.5%	9.5%
Terminal rates	3/43 (7%)	1/40 (3%)	4/42 (10%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests	P=0.402	P=0.331N	P=0.487
Logistic regression tests	P=0.402	P=0.331N	P=0.487
Cochran-Armitage test	P=0.413		
Fisher exact test		P=0.324N	P=0.500

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	15/50 (30%)	4/48 (8%)	10/50 (20%)
Adjusted rates	34.9%	10.0%	23.8%
Terminal rates	15/43 (35%)	4/40 (10%)	10/42 (24%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests	P=0.137N	P=0.008N	P=0.190N
Logistic regression tests	P=0.137N	P=0.008N	P=0.190N
Cochran-Armitage test	P=0.128N		
Fisher exact test		P=0.006N	P=0.178N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	4/50 (8%)	2/49 (4%)	3/50 (6%)
Adjusted rates	9.3%	4.7%	7.1%
Terminal rates	4/43 (9%)	1/40 (3%)	3/42 (7%)
First incidence (days)	729 (T)	607	729 (T)
Life table tests	P=0.428N	P=0.363N	P=0.513N
Logistic regression tests	P=0.419N	P=0.327N	P=0.513N
Cochran-Armitage test	P=0.417N		
Fisher exact test		P=0.349N	P=0.500N
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma			
Overall rates	3/50 (6%)	2/49 (4%)	4/50 (8%)
Adjusted rates	7.0%	4.3%	9.5%
Terminal rates	3/43 (7%)	0/40 (0%)	4/42 (10%)
First incidence (days)	729 (T)	605	729 (T)
Life table tests	P=0.409	P=0.509N	P=0.487
Logistic regression tests	P=0.414	P=0.507N	P=0.487
Cochran-Armitage test	P=0.417		
Fisher exact test		P=0.510N	P=0.500
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma			
Overall rates	5/50 (10%)	3/49 (6%)	5/50 (10%)
Adjusted rates	11.6%	6.7%	11.9%
Terminal rates	5/43 (12%)	1/40 (3%)	5/42 (12%)
First incidence (days)	729 (T)	605	729 (T)
Life table tests	P=0.557	P=0.381N	P=0.616
Logistic regression tests	P=0.568	P=0.350N	P=0.616
Cochran-Armitage test	P=0.570N		
Fisher exact test		P=0.369N	P=0.630N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rates	2/50 (4%)	6/49 (12%)	6/50 (12%)
Adjusted rates	4.7%	13.9%	13.6%
Terminal rates	2/43 (5%)	3/40 (8%)	4/42 (10%)
First incidence (days)	729 (T)	628	438
Life table tests	P=0.117	P=0.126	P=0.134
Logistic regression tests	P=0.113	P=0.141	P=0.133
Cochran-Armitage test	P=0.115		
Fisher exact test		P=0.128	P=0.134

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
All Organs: Benign Tumors			
Overall rates	15/50 (30%)	10/49 (20%)	15/50 (30%)
Adjusted rates	34.9%	25.0%	35.7%
Terminal rates	15/43 (35%)	10/40 (25%)	15/42 (36%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests	P=0.517	P=0.231N	P=0.558
Logistic regression tests	P=0.517	P=0.231N	P=0.558
Cochran-Armitage test	P=0.545		
Fisher exact test		P=0.193N	P=0.586N
All Organs: Malignant Tumors			
Overall rates	11/50 (22%)	15/49 (31%)	16/50 (32%)
Adjusted rates	25.6%	32.6%	36.3%
Terminal rates	11/43 (26%)	9/40 (23%)	14/42 (33%)
First incidence (days)	729 (T)	605	438
Life table tests	P=0.155	P=0.212	P=0.168
Logistic regression tests	P=0.150	P=0.268	P=0.175
Cochran-Armitage test	P=0.159		
Fisher exact test		P=0.228	P=0.184
All Organs: Benign or Malignant Tumors			
Overall rates	23/50 (46%)	24/49 (49%)	25/50 (50%)
Adjusted rates	53.5%	52.2%	56.8%
Terminal rates	23/43 (53%)	18/40 (45%)	23/42 (55%)
First incidence (days)	729 (T)	605	438
Life table tests	P=0.347	P=0.390	P=0.376
Logistic regression tests	P=0.367	P=0.556	P=0.407
Cochran-Armitage test	P=0.382		
Fisher exact test		P=0.462	P=0.421

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	6,250 ppm	12,500 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	4	6	2
Moribund kills	3	3	6
Survivors			
Terminal sacrifice	43	40	42
Missing		1	
Animals examined microscopically	50	49	50
Alimentary System			
Intestine large, cecum	(48)	(4)	(48)
Peyer's patch, hyperplasia, lymphoid	7 (15%)		11 (23%)
Intestine large, rectum	(48)	(7)	(47)
Serosa, inflammation, suppurative	1 (2%)		
Intestine small, ileum	(48)	(5)	(48)
Peyer's patch, hyperplasia, lymphoid		1 (20%)	1 (2%)
Intestine small, jejunum	(47)	(14)	(48)
Hyperplasia, neutrophil		1 (7%)	
Ulcer			1 (2%)
Peyer's patch, hyperplasia, lymphoid	6 (13%)	3 (21%)	8 (17%)
Serosa, inflammation, chronic active			1 (2%)
Liver	(50)	(49)	(50)
Amyloid deposition		1 (2%)	
Cyst	1 (2%)		1 (2%)
Cytomegaly, focal	4 (8%)		2 (4%)
Fatty change, diffuse	1 (2%)		
Fatty change, focal	2 (4%)		2 (4%)
Granuloma, multifocal	1 (2%)		
Hematopoietic cell proliferation		1 (2%)	2 (4%)
Infarct	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	3 (6%)	1 (2%)	
Infiltration cellular, mixed cell		1 (2%)	1 (2%)
Mitotic alteration	1 (2%)		1 (2%)
Mixed cell focus	1 (2%)		
Necrosis, focal	1 (2%)	3 (6%)	1 (2%)
Syncytial alteration	1 (2%)		
Bile duct, hyperplasia			1 (2%)
Centrilobular, cytomegaly, diffuse	1 (2%)		
Centrilobular, necrosis, diffuse	1 (2%)		
Hepatocyte, hyperplasia, focal		1 (2%)	
Portal, fibrosis			1 (2%)
Portal, inflammation, chronic	1 (2%)		
Serosa, infiltration cellular, histiocyte		1 (2%)	
Mesentery	(1)		(1)
Infiltration cellular, lymphocyte	1 (100%)		
Pancreas	(49)	(7)	(50)
Infiltration cellular, lymphocyte, multifocal	4 (8%)		1 (2%)
Inflammation, chronic active			1 (2%)
Acinus, atrophy			1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Alimentary System (continued)			
Salivary glands	(50)	(7)	(49)
Infiltration cellular, lymphocyte, multifocal	13 (26%)		10 (20%)
Stomach, forestomach	(49)	(6)	(50)
Abscess	1 (2%)		
Serosa, inflammation, chronic active			1 (2%)
Stomach, glandular	(49)	(6)	(50)
Inflammation, chronic active		1 (17%)	
Epithelium, hyperplasia, focal		1 (17%)	
Mucosa, vacuolization cytoplasmic	1 (2%)		
Tooth			(3)
Abscess			1 (33%)
Dysplasia			2 (67%)
Cardiovascular System			
Blood vessel			(1)
Aorta, inflammation, chronic active			1 (100%)
Heart	(50)	(8)	(50)
Atrium, thrombus	1 (2%)		
Myocardium, fibrosis		1 (13%)	
Myocardium, mineralization, multifocal			1 (2%)
Endocrine System			
Adrenal gland	(50)	(7)	(50)
Capsule, ectopic tissue			1 (2%)
Capsule, hyperplasia	1 (2%)		
Capsule, hyperplasia, multifocal	30 (60%)	2 (29%)	38 (76%)
Adrenal gland, cortex	(50)	(7)	(50)
Hyperplasia, focal	2 (4%)		3 (6%)
Hypertrophy, focal	6 (12%)		12 (24%)
Adrenal gland, medulla	(50)	(6)	(48)
Hyperplasia, focal			1 (2%)
Islets, pancreatic	(49)	(7)	(50)
Hyperplasia	7 (14%)	1 (14%)	2 (4%)
Pituitary gland	(48)	(7)	(44)
Pars distalis, cyst			1 (2%)
Pars distalis, hyperplasia, focal		1 (14%)	
Thyroid gland	(50)	(7)	(49)
Infiltration cellular, lymphocyte	1 (2%)	1 (14%)	
Follicle, cyst, multiple	1 (2%)		
Follicular cell, hyperplasia, focal			1 (2%)
General Body System			
None			

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Genital System			
Epididymis	(50)	(9)	(50)
Granuloma sperm	1 (2%)		
Serosa, necrosis, focal			1 (2%)
Penis	(3)	(1)	(4)
Congestion			1 (25%)
Inflammation, acute		1 (100%)	1 (25%)
Necrosis	2 (67%)		1 (25%)
Preputial gland	(6)	(7)	(11)
Abscess			4 (36%)
Cyst		1 (14%)	4 (36%)
Dilatation	4 (67%)	3 (43%)	2 (18%)
Hyperplasia	1 (17%)		
Inflammation, chronic	1 (17%)	5 (71%)	3 (27%)
Prostate	(50)	(7)	(48)
Inflammation, acute	3 (6%)		1 (2%)
Inflammation, chronic active			1 (2%)
Seminal vesicle	(49)	(9)	(50)
Fibrosis	3 (6%)	1 (11%)	
Inflammation, acute	1 (2%)		
Inflammation, chronic	2 (4%)	1 (11%)	2 (4%)
Testes	(50)	(9)	(50)
Mineralization, focal			1 (2%)
Spermatocele	1 (2%)		
Seminiferous tubule, atrophy		1 (11%)	
Seminiferous tubule, dilatation	1 (2%)		
Hematopoietic System			
Bone marrow	(50)	(8)	(50)
Hyperplasia			1 (2%)
Hyperplasia, neutrophil	2 (4%)	2 (25%)	5 (10%)
Erythroid cell, depletion			1 (2%)
Lymph node	(48)	(13)	(47)
Hyperplasia, lymphoid	2 (4%)		
Hyperplasia, plasma cell			2 (4%)
Axillary, hemorrhage	1 (2%)		
Axillary, hyperplasia, lymphoid	1 (2%)		
Iliac, hyperplasia, lymphoid	1 (2%)		
Inguinal, hyperplasia, lymphoid	1 (2%)		4 (9%)
Inguinal, hyperplasia, plasma cell		1 (8%)	1 (2%)
Inguinal, pigmentation, hemosiderin	2 (4%)		
Lumbar, hyperplasia, plasma cell			1 (2%)
Mandibular, hemorrhage			1 (2%)
Mandibular, hyperplasia, lymphoid	3 (6%)		
Mandibular, pigmentation, hemosiderin	1 (2%)		
Renal, hyperplasia, plasma cell			1 (2%)
Lymph node, mesenteric	(45)	(10)	(45)
Hematopoietic cell proliferation			1 (2%)
Hemorrhage	13 (29%)	3 (30%)	10 (22%)
Hyperplasia, lymphoid	8 (18%)	2 (20%)	9 (20%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Hematopoietic System (continued)			
Spleen	(50)	(39)	(50)
Congestion	1 (2%)		
Depletion lymphoid			2 (4%)
Dysplasia			1 (2%)
Hematopoietic cell proliferation	11 (22%)	4 (10%)	12 (24%)
Hyperplasia, lymphoid	2 (4%)	3 (8%)	1 (2%)
Pigmentation, hemosiderin	1 (2%)		1 (2%)
Thymus	(44)	(4)	(45)
Depletion lymphoid	3 (7%)	2 (50%)	7 (16%)
Epithelial cell, hyperplasia			1 (2%)
Thymocyte, necrosis	4 (9%)		
Integumentary System			
Skin	(50)	(20)	(50)
Inflammation, acute			2 (4%)
Inflammation, chronic	6 (12%)	1 (5%)	3 (6%)
Inflammation, chronic active	2 (4%)	1 (5%)	
Ulcer	5 (10%)	1 (5%)	2 (4%)
Epithelium, hyperplasia	1 (2%)	1 (5%)	
Hair follicle, atrophy	1 (2%)		
Prepuce, inflammation, acute			1 (2%)
Prepuce, ulcer			1 (2%)
Subcutaneous tissue, abscess	1 (2%)		
Subcutaneous tissue, edema	1 (2%)	1 (5%)	
Musculoskeletal System			
None			
Nervous System			
Brain	(50)	(40)	(49)
Hemorrhage		1 (3%)	
Mineralization, multifocal	38 (76%)	37 (93%)	18 (37%)
Respiratory System			
Lung	(50)	(48)	(50)
Giant cell	2 (4%)		
Leukocytosis		2 (4%)	
Alveolar epithelium, hyperplasia, focal	3 (6%)	2 (4%)	2 (4%)
Alveolus, infiltration cellular, histiocyte	2 (4%)	1 (2%)	
Artery, hypertrophy, multifocal		1 (2%)	
Bronchiole, inflammation, chronic	2 (4%)		
Interstitium, fibrosis			1 (2%)
Peribronchial, infiltration cellular, lymphocyte	2 (4%)		
Peribronchiolar, infiltration cellular, lymphocyte	1 (2%)	1 (2%)	
Perivascular, infiltration cellular, lymphocyte		1 (2%)	1 (2%)
Nose	(50)	(8)	(49)
Mucosa, degeneration, hyaline	3 (6%)		4 (8%)
Mucosa, inflammation, acute			1 (2%)
Mucosa, inflammation, chronic active	3 (6%)		
Respiratory epithelium, hyperplasia	6 (12%)	1 (13%)	5 (10%)
Trachea	(50)	(7)	(50)
Infiltration cellular, lymphocyte		1 (14%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Special Senses System			
Eye	(1)	(1)	
Conjunctiva, inflammation, chronic	1 (100%)		
Lens, cataract		1 (100%)	
Urinary System			
Kidney	(50)	(14)	(50)
Hydronephrosis			1 (2%)
Hyperplasia, atypical, focal			1 (2%)
Infarct		1 (7%)	
Infiltration cellular, lymphocyte	18 (36%)	1 (7%)	13 (26%)
Infiltration cellular, mixed cell	1 (2%)		1 (2%)
Nephropathy, chronic	8 (16%)		7 (14%)
Cortex, cyst	1 (2%)	2 (14%)	2 (4%)
Cortex, mineralization, multifocal	5 (10%)	2 (14%)	1 (2%)
Glomerulus, amyloid deposition	1 (2%)		
Pelvis, fibrosis			1 (2%)
Pelvis, inflammation, acute	1 (2%)		3 (6%)
Pelvis, inflammation, chronic active	1 (2%)		
Renal tubule, degeneration, focal	2 (4%)		3 (6%)
Renal tubule, dilatation			2 (4%)
Ureter			(1)
Hyperplasia			1 (100%)
Urethra	(2)		(2)
Inflammation, acute	1 (50%)		1 (50%)
Bulbourethral gland, ectasia	1 (50%)		
Bulbourethral gland, inflammation, acute			1 (50%)
Urinary bladder	(48)	(7)	(49)
Dilatation			2 (4%)
Infiltration cellular, lymphocyte	5 (10%)	1 (14%)	6 (12%)
Inflammation, acute	1 (2%)		1 (2%)

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF 4,4′-DIAMINO-2,2′-STILBENEDISULFONIC ACID, DISODIUM SALT

TABLE D1 Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 161

TABLE D2 Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 164

TABLE D3 Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 182

TABLE D4 Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 185

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	6,250 ppm	12,500 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	5	5	6
Moribund kills	2	2	5
Survivors			
Terminal sacrifice	43	43	38
Missing			1
Animals examined microscopically	50	50	49
Alimentary System			
Esophagus	(47)	(6)	(46)
Gallbladder	(45)	(3)	(43)
Intestine large, cecum	(48)	(4)	(45)
Intestine large, rectum	(46)	(4)	(47)
Intestine small, duodenum	(47)	(2)	(38)
Intestine small, ileum	(46)	(2)	(44)
Intestine small, jejunum	(49)	(28)	(43)
Liver	(50)	(17)	(49)
Hepatocellular carcinoma	2 (4%)		
Hepatocellular adenoma	3 (6%)		2 (4%)
Mesentery	(5)	(1)	(1)
Pancreas	(50)	(6)	(48)
Salivary glands	(50)	(6)	(47)
Stomach, forestomach	(49)	(7)	(49)
Papilloma squamous	1 (2%)		
Stomach, glandular	(50)	(6)	(48)
Cardiovascular System			
Heart	(50)	(6)	(49)
Endocrine System			
Adrenal gland	(50)	(7)	(48)
Adrenal gland, cortex	(49)	(6)	(48)
Adrenal gland, medulla	(49)	(6)	(47)
Pheochromocytoma benign	1 (2%)		
Islets, pancreatic	(50)	(5)	(48)
Adenoma		1 (20%)	
Pituitary gland	(50)	(7)	(48)
Pars distalis, adenoma	7 (14%)	2 (29%)	6 (13%)
Pars distalis, carcinoma	1 (2%)		1 (2%)
Thyroid gland	(50)	(7)	(46)
Follicular cell, adenoma		1 (14%)	
Follicular cell, carcinoma			1 (2%)
General Body System			
Tissue NOS	(1)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Genital System			
Ovary	(50)	(15)	(49)
Cystadenoma		1 (7%)	1 (2%)
Uterus	(50)	(45)	(48)
Hemangioma			1 (2%)
Mixed tumor malignant			1 (2%)
Endometrium, polyp stromal	1 (2%)		1 (2%)
Hematopoietic System			
Bone marrow	(50)	(7)	(48)
Lymph node	(48)	(17)	(46)
Lymph node, mesenteric	(48)	(5)	(46)
Spleen	(50)	(29)	(49)
Hemangiosarcoma		1 (3%)	1 (2%)
Hemangiosarcoma, metastatic, skin	1 (2%)		
Thymus	(46)	(7)	(46)
Integumentary System			
Mammary gland	(45)	(6)	(43)
Adenocarcinoma	1 (2%)		1 (2%)
Skin	(50)	(27)	(49)
Subcutaneous tissue, fibrosarcoma			1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)		
Subcutaneous tissue, schwannoma malignant		1 (4%)	
Musculoskeletal System			
Skeletal muscle			(2)
Nervous System			
Brain	(50)	(6)	(49)
Oligodendroglioma benign	1 (2%)		
Respiratory System			
Larynx	(36)		(39)
Lung	(50)	(49)	(49)
Alveolar/bronchiolar adenoma	10 (20%)	3 (6%)	4 (8%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)		1 (2%)
Alveolar/bronchiolar carcinoma	2 (4%)		
Nose	(50)	(6)	(48)
Trachea	(50)	(6)	(49)
Special Senses System			
Harderian gland		(1)	(2)
Adenoma		1 (100%)	2 (100%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Urinary System			
Kidney	(50)	(14)	(49)
Renal tubule, carcinoma	1 (2%)		
Renal tubule, carcinoma, metastatic, kidney	1 (2%)		
Urinary bladder	(49)	(6)	(47)
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(49)
Leukemia			1 (2%)
Lymphoma malignant histiocytic	2 (4%)	1 (2%)	1 (2%)
Lymphoma malignant lymphocytic	8 (16%)	4 (8%)	5 (10%)
Lymphoma malignant mixed	7 (14%)	5 (10%)	4 (8%)
Lymphoma malignant undifferentiated cell	2 (4%)		1 (2%)
Tumor Summary			
Total animals with primary neoplasms[c]	34	17	24
Total primary neoplasms	53	21	37
Total animals with benign neoplasms	21	9	12
Total benign neoplasms	26	9	18
Total animals with malignant neoplasms	24	12	15
Total malignant neoplasms	27	12	19
Total animals with secondary neoplasms	2		
Total secondary neoplasms	2		

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with tissues examined microscopically
[c] Primary tumors: all tumors except secondary tumors

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm

Number of Days on Study	410	587	602	614	659	710	714	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730
Carcass ID Number	0181	0173	0242	0163	0241	0224	0235	0124	0123	0115	0139	0194	0220	0205	0221	0222	0223	0245	0231	0133	0141	0162	0164	0172	0182
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	A	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Intestine small	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular adenoma													X												
Mesentery						+															+				+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																									X
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																			+						
Cardiovascular System																									
Blood vessel			+																						
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																		X							
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	M	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	M
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																					X				
Pars distalis, carcinoma																									X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0183	0212	0223	0244	0135	0145	0154	0155	0174	0185	0192	0211	0224	0225	0231	0244	0253	0261	0291	0295	0201	0203	0215	0223	0233	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	47
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	46
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																		X	X							2
Hepatocellular adenoma		X																					X			3
Mesentery													+								+					5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Papilloma squamous																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	M	M	M	+	+	M	+	+	M	+	+	+	M	+	+	M	+	+	M	+	+	M	35
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X		X	X									X							X	X					7
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	410	587	602	614	695	710	714	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730
Carcass ID Number	01812	01713	02142	01163	02241	02242	02345	01142	01143	01151	01193	01194	02202	02205	02221	02222	02223	02245	01131	01133	01141	01162	01164	01172	01182
General Body System																									
Tissue NOS						+																			
Genital System																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrium, polyp stromal																									X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma, metastatic, skin																									
Thymus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma		X																							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, hemangiosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oligodendroglioma benign	X																								
Respiratory System																									
Larynx							+	+	M	+	M	+	M	+	+	+	+	+		+	+	M	+	M	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma							X			X							X	X							X
Alveolar/bronchiolar adenoma, multiple													X												
Alveolar/bronchiolar carcinoma																	X								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732		
Carcass ID Number	018	021	024	024	013	014	015	015	017	018	019	011	012	022	031	044	053	061	091	095	001	023	015	023		Total Tissues/ Tumors
General Body System																										
Tissue NOS																										1
Genital System																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrium, polyp stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma, metastatic, skin	X																									1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	46
Integumentary System																										
Mammary gland	+	M	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Adenocarcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, hemangiosarcoma	X																									1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma benign																										1
Respiratory System																										
Larynx	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	36
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma								X											X	X		X			X	10
Alveolar/bronchiolar adenoma, multiple									X																	2
Alveolar/bronchiolar carcinoma																					X					2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	410	508	602	614	695	710	714	729	229	229	229	229	229	229	229	229	229	330	330	330	330	330	330	330
Carcass ID Number	018	017	024	016	124	222	233	141	143	151	139	194	202	205	221	222	223	245	131	133	141	162	164	172	182
Special Senses System																									
None																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, carcinoma			X																						
Renal tubule, carcinoma, metastatic, kidney			X																						
Urinary bladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic		X				X																			
Lymphoma malignant lymphocytic											X		X			X				X	X				
Lymphoma malignant mixed				X			X																X		
Lymphoma malignant undifferentiated cell type					X									X											

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 0 ppm (continued)

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732		
Carcass ID Number	0128	0212	0224	0244	0133	0145	0154	0155	0174	0185	0192	0211	0224	0225	0231	0244	0115	0116	0119	0195	0120	0220	0221	0223		Total Tissues/ Tumors
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Renal tubule, carcinoma																										1
Renal tubule, carcinoma, metastatic, kidney																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Lymphoma malignant histiocytic																										2
Lymphoma malignant lymphocytic							X											X					X			8
Lymphoma malignant mixed				X	X			X													X					7
Lymphoma malignant undifferentiated cell type																										2

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm

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

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm (continued)

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0445	0444	0455	0447	0337	0338	0339	0401	0405	0413	0422	0443	0442	0444	0448	0437	0437	0437	0438	0439	0439	0445	0446	0448	0448	Total Tissues/ Tumors
Alimentary System																										
Esophagus																										6
Gallbladder																										3
Intestine large																										6
Intestine large, cecum																										4
Intestine large, colon																										4
Intestine large, rectum																										4
Intestine small	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	28
Intestine small, duodenum																										2
Intestine small, ileum																										2
Intestine small, jejunum	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	28
Liver						+	+		+			+	+				+				+			+	+	17
Mesentery																										1
Pancreas							+																			6
Salivary glands																										6
Stomach																										7
Stomach, forestomach																										7
Stomach, glandular																										6
Tongue							+																			1
Cardiovascular System																										
Heart																										6
Endocrine System																										
Adrenal gland																										7
Adrenal gland, cortex																										6
Adrenal gland, medulla																										6
Islets, pancreatic																										5
Adenoma																										1
Parathyroid gland																										
Pituitary gland	+																									7
Pars distalis, adenoma	X																									2
Thyroid gland							+																			7
Follicular cell, adenoma																										1
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm (continued)

Number of Days on Study	1	4	5	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	6	3	5	0	3	6	7	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3
	3	8	8	1	9	2	6	9	9	9	9	9	9	9	9	9	9	9	0	0	0	0	0	0	0
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	4	4	4	4	4	4	3	3	3	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4
	7	5	3	3	5	6	2	7	8	9	0	1	1	1	2	3	3	8	9	0	0	1	2	4	4
	1	1	5	3	3	3	1	1	4	5	2	1	2	5	3	1	2	2	1	3	4	4	5	1	3
Genital System																									
Ovary	+	A	+	+	+	+	+				+	+		+			+		+	+					
Cystadenoma																			X						
Uterus	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+																		
Lymph node	M	A	+	+	+	+	+				+							+					+	+	
Lymph node, mesenteric	M	A	M	+	+	+	+																		
Spleen	M	A	+	+	+	+	+	+	+	+					+		+					+		+	
Hemangiosarcoma																									
Thymus	M	M	+	+	+	+	+								+										
Integumentary System																									
Mammary gland	+	M	+	+	+	+	+																		
Skin	+	+	+	+	+	+	M	+	+		+		+						+	+	+	+	+		
Subcutaneous tissue, schwannoma malignant					X																				
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+																		
Nervous System																									
Brain	M	+	+	+	+	+	+																		
Respiratory System																									
Lung	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																		X							
Nose	M	+	+	+	+	+	+																		
Trachea	M	+	+	+	+	+	+																		
Special Senses System																									
Eye																									+
Harderian gland																									+
Adenoma																									X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm (continued)

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0445	0445	0455	0473	0372	0385	0393	0401	0405	0413	0422	0434	0442	0444	0484	0373	0374	0375	0382	0392	0394	0452	0465	0483	0485	Total Tissues/ Tumors
Genital System																										
Ovary		+					+															+				15
Cystadenoma																										1
Uterus		+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Hematopoietic System																										
Bone marrow																										7
Lymph node			+				+						+			+	+	+	+	+						17
Lymph node, mesenteric							+																			5
Spleen	+					+	+	+	+	+	+	+	+		+	+		+			+	+	+	+	+	29
Hemangiosarcoma											X															1
Thymus							+																			7
Integumentary System																										
Mammary gland																										6
Skin	+		+	+	+	+	+	+	+						+			+			+				+	27
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone																										7
Nervous System																										
Brain																										6
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma				X														X								3
Nose																										6
Trachea																										6
Special Senses System																										
Eye																										1
Harderian gland																										1
Adenoma																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm (continued)

Number of Days on Study	163	438	558	601	639	662	676	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730
Carcass ID Number	0471	0451	0443	0443	0445	0446	0442	0337	0338	0339	0440	0441	0441	0445	0443	0443	0438	0439	0340	0440	0444	0445	0441	0443	0441	0443
Urinary System																										
Kidney	+	A		+	+	+	+		+																	
Urinary bladder	+	+	+	+	+	+	A																			
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																										
Lymphoma malignant lymphocytic						X									X											
Lymphoma malignant mixed				X																		X		X		

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 6,250 ppm (continued)

Number of Days on Study	730	730	730	730	731	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0445	0454	0455	0473	0372	0385	0393	0401	0405	0413	0422	0434	0442	0444	0484	0373	0377	0375	0382	0392	0394	0452	0462	0485	0485	Total Tissues/ Tumors
Urinary System																										
Kidney						+	+				+				+			+	+		+			+		14
Urinary bladder																										6
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic								X																		1
Lymphoma malignant lymphocytic													X				X									4
Lymphoma malignant mixed						X	X																			5

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm

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

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	
Carcass ID Number	065 2	065 3	066 3	067 1	067 4	069 5	072 5	063 1	066 2	069 2	070 2	077 3	077 3	071 5	077 2	077 3	061 5	062 3	066 5	066 7	068 1	070 1	071 1	071 2	071 4	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	46
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	43
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, duodenum	+	M	+	+	M	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	38
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma								X			X															2
Mesentery																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	M	+	M	+	+	+	M	M	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	M	32
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma											X			X				X								6
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Follicular cell, carcinoma																										1
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	529	602	639	640	660	667	672	680	684	718	721	729	729	729	729	729	729	729	729	729	730	730	730
Carcass ID Number	0633	0683	0621	0662	0613	0705	0704	0651	0644	0648	0653	0611	0624	0643	0644	0655	0661	0673	0682	0685	0721	0614	0623	0634
Genital System																								
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																								
Uterus	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																								
Mixed tumor malignant																								
Endometrium, polyp stromal										X														
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																								
Thymus	+	M	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M
Adenocarcinoma				X																				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma										X														
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle											+										+			
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord					+																			
Respiratory System																								
Larynx										+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma										X	X													
Alveolar/bronchiolar adenoma, multiple																								
Nose	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0652	0653	0663	0671	0674	0695	0725	0763	0766	0769	0770	0773	0771	0775	0772	0773	0765	0762	0765	0767	0781	0770	0771	0772	0774	Total Tissues/ Tumors
Genital System																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cystadenoma						X																				1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangioma																		X								1
Mixed tumor malignant																								X		1
Endometrium, polyp stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangiosarcoma														X												1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	46
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	43
Adenocarcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Subcutaneous tissue, fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Skeletal muscle																										2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spinal cord																										1
Respiratory System																										
Larynx	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	39
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma							X	X																		4
Alveolar/bronchiolar adenoma, multiple						X																				1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	529	602	639	640	660	667	672	680	684	718	721	729	729	729	729	729	729	729	729	729	730	730	730
Carcass ID Number	0633	0683	0621	0663	0661	0707	0704	0765	0764	0768	0763	0761	0762	0764	0766	0765	0761	0767	0768	0768	0721	0761	0762	0763
Special Senses System																								
Ear																								
Eye											+													
Harderian gland																								
Adenoma																								
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia		X																						
Lymphoma malignant histiocytic																								
Lymphoma malignant lymphocytic													X		X									
Lymphoma malignant mixed			X					X		X	X													
Lymphoma malignant undifferentiated cell type							X																	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt: 12,500 ppm (continued)

Number of Days on Study	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	
Carcass ID Number	0652	0653	0663	0671	0674	0695	0725	0763	0762	0769	0770	0770	0771	0771	0772	0773	0761	0762	0766	0767	0768	0770	0771	0771	0774	Total Tissues/ Tumors
Special Senses System																										
Ear																							+			1
Eye																									+	2
Harderian gland								+																	+	2
Adenoma								X																	X	2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	47
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia																										1
Lymphoma malignant histiocytic									X																	1
Lymphoma malignant lymphocytic																X						X	X			5
Lymphoma malignant mixed																										4
Lymphoma malignant undifferentiated cell type																										1

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm	6,250 ppm	12,500 ppm
Liver: Hepatocellular Adenoma			
Overall rates[a]	3/50 (6%)	0/17 (0%)[e]	2/49 (4%)
Adjusted rates[b]	7.0%		5.3%
Terminal rates[c]	3/43 (7%)		2/38 (5%)
First incidence (days)	729 (T)		729 (T)
Life table tests[d]			P=0.556N
Logistic regression tests[d]			P=0.556N
Fisher exact test[d]			P=0.510N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates	5/50 (10%)	0/17 (0%)[e]	2/49 (4%)
Adjusted rates	11.6%		5.3%
Terminal rates	5/43 (12%)		2/38 (5%)
First incidence (days)	729 (T)		729 (T)
Life table tests			P=0.269N
Logistic regression tests			P=0.269N
Fisher exact test			P=0.226N
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	12/50 (24%)	3/49 (6%)	5/49 (10%)
Adjusted rates	27.3%	7.0%	12.5%
Terminal rates	11/43 (26%)	3/43 (7%)	3/38 (8%)
First incidence (days)	714	729 (T)	718
Life table tests	P=0.046N	P=0.013N	P=0.096N
Logistic regression tests	P=0.038N	P=0.014N	P=0.077N
Cochran-Armitage test[d]	P=0.031N		
Fisher exact test		P=0.013N	P=0.059N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	13/50 (26%)	3/49 (6%)	5/49 (10%)
Adjusted rates	29.5%	7.0%	12.5%
Terminal rates	12/43 (28%)	3/43 (7%)	3/38 (8%)
First incidence (days)	714	729 (T)	718
Life table tests	P=0.027N	P=0.007N	P=0.065N
Logistic regression tests	P=0.022N	P=0.008N	P=0.050N
Cochran-Armitage test	P=0.017N		
Fisher exact test		P=0.007N	P=0.037N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	7/50 (14%)	2/7 (29%)[e]	6/48 (13%)
Adjusted rates	16.3%		15.1%
Terminal rates	7/43 (16%)		5/38 (13%)
First incidence (days)	729 (T)		660
Life table tests			P=0.588N
Logistic regression tests			P=0.546N
Fisher exact test			P=0.532N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rates	8/50 (16%)	2/7 (29%)[e]	7/48 (15%)
Adjusted rates	18.6%		17.7%
Terminal rates	8/43 (19%)		6/38 (16%)
First incidence (days)	729 (T)		660
Life table tests			P=0.597N
Logistic regression tests			P=0.553N
Fisher exact test			P=0.535N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rates	19/50 (38%)	10/50 (20%)	11/49 (22%)
Adjusted rates	39.6%	22.1%	25.3%
Terminal rates	14/43 (33%)	8/43 (19%)	6/38 (16%)
First incidence (days)	587	601	639
Life table tests	P=0.097N	P=0.052N	P=0.140N
Logistic regression tests	P=0.051N	P=0.040N	P=0.071N
Cochran-Armitage test	P=0.050N		
Fisher exact test		P=0.038N	P=0.071N
All Organs: Benign Tumors			
Overall rates	21/50 (42%)	9/50 (18%)	12/49 (24%)
Adjusted rates	46.5%	19.9%	29.1%
Terminal rates	19/43 (44%)	7/43 (16%)	9/38 (24%)
First incidence (days)	410	601	660
Life table tests	P=0.065N	P=0.010N	P=0.103N
Logistic regression tests	P=0.034N	P=0.009N	P=0.052N
Cochran-Armitage test	P=0.033N		
Fisher exact test		P=0.008N	P=0.051N
All Organs: Malignant Tumors			
Overall rates	24/50 (48%)	12/50 (24%)	15/49 (31%)
Adjusted rates	49.0%	26.0%	33.0%
Terminal rates	18/43 (42%)	9/43 (21%)	8/38 (21%)
First incidence (days)	587	601	602
Life table tests	P=0.094N	P=0.020N	P=0.137N
Logistic regression tests	P=0.042N	P=0.011N	P=0.056N
Cochran-Armitage test	P=0.042N		
Fisher exact test		P=0.011N	P=0.059N
All Organs: Benign or Malignant Tumors			
Overall rates	34/50 (68%)	17/50 (34%)	24/49 (49%)
Adjusted rates	68.0%	36.9%	51.9%
Terminal rates	27/43 (63%)	14/43 (33%)	16/38 (42%)
First incidence (days)	410	601	602
Life table tests	P=0.110N	P=0.002N	P=0.157N
Logistic regression tests	P=0.035N	P<0.001N	P=0.035N
Cochran-Armitage test	P=0.035N		
Fisher exact test		P<0.001N	P=0.043N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus, statistical comparisons with the control are not appropriate.

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	6,250 ppm	12,500 ppm
Disposition Summary			
Animals initially in study	60	60	60
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	5	5	6
Moribund kills	2	2	5
Survivors			
Terminal sacrifice	43	43	38
Missing			1
Animals examined microscopically	50	50	49
Alimentary System			
Gallbladder	(45)	(3)	(43)
Infiltration cellular, lymphocyte	1 (2%)		
Intestine large, cecum	(48)	(4)	(45)
Peyer's patch, hyperplasia, lymphoid	5 (10%)		5 (11%)
Intestine small, jejunum	(49)	(28)	(43)
Hemorrhage			1 (2%)
Peyer's patch, angiectasis		1 (4%)	
Peyer's patch, hyperplasia, lymphoid	5 (10%)	3 (11%)	4 (9%)
Liver	(50)	(17)	(49)
Basophilic focus, multiple	1 (2%)		
Cytoplasmic alteration, focal		1 (6%)	1 (2%)
Fatty change, diffuse	1 (2%)	1 (6%)	2 (4%)
Fatty change, focal	1 (2%)		
Granuloma	1 (2%)	1 (6%)	
Hematocyst			1 (2%)
Hematopoietic cell proliferation	1 (2%)	1 (6%)	1 (2%)
Infarct		1 (6%)	
Infiltration cellular, lymphocyte	10 (20%)	2 (12%)	4 (8%)
Mitotic alteration	1 (2%)		
Necrosis, focal	3 (6%)	2 (12%)	4 (8%)
Pigmentation, lipofuscin		1 (6%)	
Bile duct, hyperplasia, multifocal	1 (2%)		
Centrilobular, fatty change, diffuse	1 (2%)		
Periportal, fatty change, diffuse	2 (4%)		
Mesentery	(5)	(1)	(1)
Amyloid deposition	1 (20%)		
Fat, necrosis, focal	2 (40%)	1 (100%)	
Pancreas	(50)	(6)	(48)
Amyloid deposition		1 (17%)	
Infiltration cellular, lymphocyte, multifocal	12 (24%)		8 (17%)
Acinus, atrophy		1 (17%)	1 (2%)
Artery, inflammation, chronic	1 (2%)		
Duct, cyst		1 (17%)	
Salivary glands	(50)	(6)	(47)
Infiltration cellular, lymphocyte, multifocal	21 (42%)	1 (17%)	20 (43%)
Stomach, forestomach	(49)	(7)	(49)
Diverticulum		1 (14%)	
Epithelium, hyperplasia, focal	2 (4%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Alimentary System (continued)			
Stomach, glandular	(50)	(6)	(48)
Hemorrhage		1 (17%)	
Infiltration cellular, lymphocyte			1 (2%)
Mineralization, focal	1 (2%)		
Necrosis, multifocal			1 (2%)
Cardiovascular System			
Blood vessel	(1)		
Artery, inflammation, chronic active	1 (100%)		
Heart	(50)	(6)	(49)
Bacterium			1 (2%)
Infiltration cellular, lymphocyte			1 (2%)
Aortic valve, thrombus			1 (2%)
Arteriole, thrombus, multifocal	1 (2%)		
Artery, hypertrophy, multifocal			1 (2%)
Mitral valve, inflammation, acute			1 (2%)
Myocardium, degeneration	1 (2%)		
Ventricle, thrombus	1 (2%)		
Endocrine System			
Adrenal gland	(50)	(7)	(48)
Capsule, ectopic tissue	4 (8%)		1 (2%)
Capsule, hyperplasia, multifocal	50 (100%)	5 (71%)	48 (100%)
Adrenal gland, cortex	(49)	(6)	(48)
Atrophy	1 (2%)		
Degeneration, fatty, multifocal	1 (2%)		
Hematopoietic cell proliferation	2 (4%)	1 (17%)	
Hemorrhage			1 (2%)
Hyperplasia, focal	3 (6%)		3 (6%)
Hypertrophy, focal	1 (2%)		1 (2%)
Adrenal gland, medulla	(49)	(6)	(47)
Hyperplasia, focal	1 (2%)		
Islets, pancreatic	(50)	(5)	(48)
Hyperplasia, multifocal	2 (4%)		1 (2%)
Parathyroid gland	(35)		(32)
Hyperplasia			1 (3%)
Infiltration cellular, lymphocyte	1 (3%)		
Pituitary gland	(50)	(7)	(48)
Pars distalis, angiectasis	3 (6%)		1 (2%)
Pars distalis, hyperplasia, focal	1 (2%)		3 (6%)
Pars distalis, hypertrophy, focal			1 (2%)
Thyroid gland	(50)	(7)	(46)
Infiltration cellular, lymphocyte	2 (4%)		1 (2%)
Inflammation, acute			1 (2%)
Follicle, cyst, multiple			1 (2%)
Follicular cell, hyperplasia, focal	3 (6%)		2 (4%)
General Body System			
None			

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Genital System			
Ovary	(50)	(15)	(49)
Abscess			1 (2%)
Cyst	16 (32%)	7 (47%)	20 (41%)
Hemorrhage			1 (2%)
Infiltration cellular, lymphocyte	3 (6%)		1 (2%)
Pigmentation, hemosiderin		1 (7%)	
Uterus	(50)	(45)	(48)
Angiectasis	2 (4%)		
Dilatation	7 (14%)	5 (11%)	3 (6%)
Hyperplasia, cystic	44 (88%)	41 (91%)	39 (81%)
Infiltration cellular, lymphocyte	1 (2%)		
Hematopoietic System			
Bone marrow	(50)	(7)	(48)
Atrophy, focal	2 (4%)		2 (4%)
Hyperplasia, neutrophil		1 (14%)	2 (4%)
Lymph node	(48)	(17)	(46)
Hyperplasia, lymphoid	1 (2%)		
Iliac, hemorrhage			1 (2%)
Iliac, hyperplasia, histiocytic			1 (2%)
Iliac, hyperplasia, lymphoid	1 (2%)	2 (12%)	1 (2%)
Inguinal, hyperplasia, lymphoid	1 (2%)		1 (2%)
Mandibular, hyperplasia, lymphoid	2 (4%)	1 (6%)	2 (4%)
Mandibular, pigmentation, hemosiderin	1 (2%)		
Mediastinal, hyperplasia, lymphoid	1 (2%)	1 (6%)	2 (4%)
Mediastinal, mineralization		1 (6%)	
Renal, hemorrhage			1 (2%)
Renal, hyperplasia, lymphoid		1 (6%)	1 (2%)
Lymph node, mesenteric	(48)	(5)	(46)
Abscess			1 (2%)
Amyloid deposition	1 (2%)		
Hemorrhage		2 (40%)	1 (2%)
Hyperplasia, lymphoid	5 (10%)		1 (2%)
Spleen	(50)	(29)	(49)
Ectopic tissue		1 (3%)	
Hematopoietic cell proliferation	4 (8%)	3 (10%)	4 (8%)
Hyperplasia, lymphoid	4 (8%)	1 (3%)	1 (2%)
Capsule, inflammation, chronic			1 (2%)
Thymus	(46)	(7)	(46)
Amyloid deposition	1 (2%)		
Depletion lymphoid	4 (9%)	3 (43%)	4 (9%)
Ectopic parathyroid gland			2 (4%)
Hyperplasia, lymphoid	4 (9%)		6 (13%)
Integumentary System			
Mammary gland	(45)	(6)	(43)
Hyperplasia	2 (4%)		2 (5%)
Inflammation, chronic		1 (17%)	

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Integumentary System (continued)			
Skin	(50)	(27)	(49)
Inflammation, acute			1 (2%)
Inflammation, chronic	21 (42%)	13 (48%)	13 (27%)
Ulcer	1 (2%)		1 (2%)
Epithelium, hyperplasia	2 (4%)	2 (7%)	
Epithelium, mitotic alteration		1 (4%)	
Subcutaneous tissue, inflammation, acute	1 (2%)		
Subcutaneous tissue, mineralization, focal		1 (4%)	
Musculoskeletal System			
Bone	(50)	(7)	(49)
Fibrous osteodystrophy	6 (12%)	1 (14%)	9 (18%)
Hyperplasia	1 (2%)		
Coccygeal, fracture			1 (2%)
Skeletal muscle			(2)
Infiltration cellular, lymphocyte			1 (50%)
Nervous System			
Brain	(50)	(6)	(49)
Hemorrhage		1 (17%)	
Mineralization, multifocal	34 (68%)	1 (17%)	31 (63%)
Arteriole, meninges, bacterium			1 (2%)
Arteriole, meninges, thrombus			1 (2%)
Meninges, inflammation, acute			1 (2%)
Respiratory System			
Lung	(50)	(49)	(49)
Atelectasis, focal	1 (2%)		
Hemorrhage, focal	1 (2%)		2 (4%)
Infiltration cellular, lymphocyte	1 (2%)		
Alveolar epithelium, hyperplasia, focal	3 (6%)	5 (10%)	
Alveolus, edema			1 (2%)
Alveolus, infiltration cellular, histiocyte	3 (6%)		1 (2%)
Artery, hypertrophy, multifocal	10 (20%)	8 (16%)	21 (43%)
Artery, mineralization, multifocal		1 (2%)	
Artery, thrombus	1 (2%)	1 (2%)	
Interstitium, inflammation, acute			1 (2%)
Mediastinum, infiltration cellular, lymphocyte			1 (2%)
Peribronchial, infiltration cellular, lymphocyte	1 (2%)	2 (4%)	
Peribronchiolar, infiltration cellular, lymphocyte	4 (8%)	1 (2%)	3 (6%)
Perivascular, infiltration cellular, lymphocyte	16 (32%)	13 (27%)	22 (45%)
Pleura, infiltration cellular, lymphocyte	1 (2%)	1 (2%)	1 (2%)
Nose	(50)	(6)	(48)
Lumen, foreign body	4 (8%)		
Mucosa, degeneration, hyaline	28 (56%)	4 (67%)	27 (56%)
Mucosa, inflammation, chronic	2 (4%)		
Mucosa, inflammation, chronic active	1 (2%)		
Nasolacrimal duct, inflammation, chronic			1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

	0 ppm	6,250 ppm	12,500 ppm
Respiratory System (continued)			
Nose (continued)			
Nasolacrimal duct, inflammation, chronic active	1 (2%)		
Respiratory epithelium, hyperplasia	11 (22%)	1 (17%)	9 (19%)
Lumen, foreign body	4 (8%)		
Mucosa, degeneration, hyaline	28 (56%)	4 (67%)	27 (56%)
Mucosa, inflammation, chronic	2 (4%)		
Mucosa, inflammation, chronic active	1 (2%)		
Nasolacrimal duct, inflammation, chronic			1 (2%)
Nasolacrimal duct, inflammation, chronic active	1 (2%)		
Respiratory epithelium, hyperplasia	11 (22%)	1 (17%)	9 (19%)
Special Senses System			
Eye		(1)	(2)
Cornea, inflammation, chronic		1 (100%)	
Cornea, inflammation, chronic active			1 (50%)
Urinary System			
Kidney	(50)	(14)	(49)
Cyst	1 (2%)		
Embolus bacterial			1 (2%)
Hydronephrosis	1 (2%)		1 (2%)
Infiltration cellular, lymphocyte	14 (28%)	5 (36%)	9 (18%)
Inflammation, acute, multifocal			1 (2%)
Inflammation, chronic, multifocal	1 (2%)		
Metaplasia, osseous		1 (7%)	
Glomerulus, amyloid deposition	3 (6%)		
Glomerulus, inflammation, chronic			1 (2%)
Renal tubule, degeneration, focal	4 (8%)	1 (7%)	
Urinary bladder	(49)	(6)	(47)
Hemorrhage			1 (2%)
Infiltration cellular, lymphocyte	20 (41%)	2 (33%)	21 (45%)

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX E
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

SALMONELLA PROTOCOL

Testing was performed as reported by Haworth *et al.* (1983) and Zeiger *et al.* (1987). 4,4′-Diamino-2,2′-stilbenedisulfonic acid was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains (TA100, TA1535, TA1537, or TA98) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C prior to the addition of soft agar supplemented with *l*-histidine and *d*-biotin, and subsequent plating on minimal glucose agar plates. Incubation continued for an additional 48 hours.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of 4,4′-diamino-2,2′-stilbenedisulfonic acid. High dose was limited to 5,000 μg/mL. All assays were repeated.

In this assay, 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 was not dose-related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment.

CHINESE HAMSTER OVARY CELL CYTOGENETICS ASSAYS

Testing was performed as reported by Galloway *et al.* (1985, 1987) and Loveday *et al.* (1990). 4,4′-Diamino-2,2′-stilbenedisulfonic acid was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCE) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each trial consisted of concurrent solvent and positive controls and of at least three doses of 4,4′-diamino-2,2′-stilbenedisulfonic acid; the high dose was limited by toxicity or solubility, but did not exceed 5,000 μg per mL.

In the SCE test without S9, CHO cells were incubated for 26 hours with 4,4′-diamino-2,2′-stilbenedisulfonic acid 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 4,4′-diamino-2,2′-stilbenedisulfonic acid was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 to 3 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with the chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no 4,4′-diamino-2,2′-stilbenedisulfonic acid and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 to 3 hours. Harvesting and staining procedures were the same as for cells treated without S9.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with 4,4′-diamino-2,2′-stilbenedisulfonic acid for 8 to 10 hours; Colcemid was added and incubation continued for 2 to 3 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with 4,4′-diamino-2,2′-stilbenedisulfonic acid and S9 for 2 hours, after which

the treatment medium was removed and the cells incubated for 10 hours in fresh medium, with Colcemid present for the final 2 to 3 hours. Cells were harvested in the the same manner as for the treatment without S9.

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

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were scored for frequency of SCE per cell from each dose level; 200 first-division metaphase cells were scored at each dose level for the Abs test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. Abs data are presented as percentage of cells with aberrations. As with SCE data, both the dose-response curve and individual dose points were statistically analyzed. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) was considered weak evidence for a positive response (+w); significant differences for two or more doses indicated the trial was positive (+) (Galloway *et al.*, 1987).

RESULTS

4,4′-Diamino-2,2′-stilbenedisulfonic acid was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 when tested in a preincubation protocol at concentrations of 100 to 5,000-μg/plate in the presence and the absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table E1; Zeiger *et al.*, 1987). 4,4′-Diamino-2,2′-stilbenedisulfonic acid was tested for induction of sister chromatid exchanges (Table E2) and chromosomal aberrations (Table E3) in Chinese hamster ovary cells in two laboratories; results in both laboratories were negative for each endpoint. In the first laboratory, 4,4′-diamino-2,2′-stilbenedisulfonic acid was tested for induction of SCE and Abs using standard harvest times, with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9, at concentrations up to 1,020 μg/mL (Loveday *et al.*, 1990). In the second laboratory, higher doses, up to 5,000 μg/mL 4,4′-diamino-2,2′-stilbenedisulfonic acid were tested with and without S9; a delayed harvest protocol was used to obtain sufficient cells for analysis at the highest dose in the SCE trials and in the Abs trial conducted in the absence of S9.

TABLE E1
Mutagenicity of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	130 ± 12.8	126 ± 9.4	146 ± 3.3	143 ± 1.7	136 ± 5.6	132 ± 11.9
	100	124 ± 4.5	120 ± 6.9	142 ± 1.9	136 ± 1.5	128 ± 3.7	126 ± 11.6
	333	138 ± 3.5	163 ± 30.4	151 ± 6.7	146 ± 6.7	132 ± 5.5	142 ± 4.4
	1,000	137 ± 9.0	134 ± 1.2	150 ± 12.1	141 ± 5.8	148 ± 7.8	143 ± 5.6
	3,333	120 ± 0.7	138 ± 3.8	140 ± 5.9	147 ± 8.0	125 ± 3.8	144 ± 7.1
	5,000	138 ± 11.4	150 ± 4.9	152 ± 6.7	126 ± 8.0	148 ± 8.2	156 ± 8.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		864 ± 0.9	1,053 ± 36.7	949 ± 23.2	1,014 ± 18.6	888 ± 17.0	775 ± 44.7
TA1535	0	22 ± 3.7	34 ± 1.8	14 ± 2.3	19 ± 0.9	16 ± 1.5	16 ± 1.5
	100	24 ± 3.5	25 ± 1.7	15 ± 2.3	26 ± 2.8	19 ± 3.0	19 ± 3.2
	333	22 ± 4.1	24 ± 2.5	19 ± 1.8	17 ± 3.4	21 ± 1.5	18 ± 5.2
	1,000	22 ± 2.0	27 ± 3.1	17 ± 2.7	19 ± 2.0	22 ± 2.8	17 ± 2.9
	3,333	22 ± 2.7	27 ± 4.6	18 ± 4.1	21 ± 1.8	22 ± 2.3	16 ± 2.5
	5,000	24 ± 2.9	24 ± 2.6	16 ± 3.8	15 ± 1.5	20 ± 1.9	26 ± 2.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		719 ± 6.4	847 ± 15.0	49 ± 7.9	89 ± 12.7	50 ± 3.2	99 ± 16.0
TA1537	0	7 ± 1.2	6 ± 1.5	7 ± 2.0	7 ± 0.6	8 ± 1.0	5 ± 0.3
	100	4 ± 0.9	4 ± 1.7	5 ± 1.2	5 ± 2.0	7 ± 2.1	3 ± 0.3
	333	6 ± 1.5	3 ± 0.9	4 ± 0.6	6 ± 2.8	6 ± 1.3	8 ± 1.7
	1,000	8 ± 0.7	8 ± 0.7	5 ± 1.9	4 ± 1.5	7 ± 2.5	6 ± 1.2
	3,333	3 ± 0.6	5 ± 0.3	6 ± 1.2	8 ± 1.8	4 ± 1.2	8 ± 0.9
	5,000	6 ± 2.7	4 ± 1.2	8 ± 2.8	6 ± 1.3	7 ± 0.7	7 ± 0.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		255 ± 3.2	312 ± 69.5	59 ± 7.8	95 ± 18.3	54 ± 2.3	41 ± 5.7

TABLE E1
Mutagenicity of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3
TA98	0	17 ± 1.5	20 ± 0.3	18 ± 2.3	32 ± 4.0	28 ± 3.5	31 ± 4.3
	100	21 ± 2.0			28 ± 2.4		
	333	19 ± 1.9		18 ± 3.8	24 ± 1.2		25 ± 0.7
	1,000	22 ± 1.5		21 ± 0.9	32 ± 2.1		30 ± 2.3
	3,000		19 ± 1.5			41 ± 5.2	
	3,333	18 ± 1.2		21 ± 2.6	37 ± 0.9		29 ± 2.9
	3,500		20 ± 1.5			36 ± 1.9	
	4,000		19 ± 1.9	25 ± 0.9		40 ± 2.7	34 ± 3.8
	4,500		17 ± 1.5	27 ± 3.0		33 ± 2.3	36 ± 5.7
	5,000	20 ± 1.9	18 ± 1.9	24 ± 2.4	61 ± 2.6	35 ± 2.0	38 ± 0.9
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Negative
Positive control		1,236 ± 24.8	1,430 ± 72.1	1,221 ± 5.0	875 ± 14.5	1,241 ± 88.1	1,023 ± 26.4

TA98 (continued)

		+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
	0	26 ± 3.5	30 ± 2.2	29 ± 1.2
	100	25 ± 1.7		
	333	27 ± 0.3		30 ± 7.2
	1,000	28 ± 3.2		23 ± 2.5
	3,000		29 ± 2.1	
	3,333	30 ± 0.6		34 ± 1.5
	3,500		29 ± 1.9	
	4,000		34 ± 2.4	40 ± 4.0
	4,500		36 ± 2.3	33 ± 3.3
	5,000	34 ± 1.9	40 ± 4.1	32 ± 5.9
Trial summary		Negative	Negative	Negative
Positive control		911 ± 49.5	831 ± 20.5	411 ± 17.5

[a] Study performed at EG&G Mason Research Institute. The detailed protocol and these data are presented in Zeiger *et al.* (1987).
[b] Revertants are presented as mean ± standard error from three plates.
[c] 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 4,4′-Diamino-2,2′-stilbenedisulfonic Acid[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/Chromosome	SCEs/Cell	Hrs in BrdU	Relative SCEs/Chromosome (%)[b]
Study performed at Bioassay Systems Corporation								
-S9								
Trial 1								
Summary: Negative								
Medium		50	1,044	340	0.32	6.8	26.5	
Mitomycin-C	0.0015	50	1,044	448	0.42	9.0	26.5	31.76
	0.0100	10	210	210	0.95	20.1	26.5	193.90
4,4′-Diamino-2,2′-stilbenedisulfonic acid								
	102	50	1,047	344	0.32	6.9	26.5	0.88
	306	50	1,037	328	0.31	6.6	26.5	−2.88
	1,020	50	1,044	306	0.29	6.1	26.5	−10.00
								P=0.922[c]
+S9								
Trial 1								
Summary: Negative								
Medium		50	1,041	346	0.33	6.9	26.0	
Cyclophosphamide	0.5	50	1,047	561	0.53	11.2	26.0	61.21
	2.5	10	208	324	1.55	32.4	26.0	368.66
4,4′-Diamino-2,2′-stilbenedisulfonic acid								
	102	50	1,040	305	0.29	6.1	26.0	−11.77
	306	50	1,041	359	0.34	7.2	26.0	3.76
	1,020	50	1,041	318	0.30	6.4	26.0	−8.09
								P=0.657

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 4,4′-Diamino-2,2′-stilbenedisulfonic Acid (continued)

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/Chromosome	SCEs/Cell	Hrs in BrdU	Relative SCEs/Chromosome (%)
Study performed at Sitek Research Laboratories								
-S9								
Trial 1								
Summary: Negative								
Distilled water		50	1,051	400	0.38	8.0	26.0	
		50	1,050	410	0.39	8.2	31.0[d]	
Mitomycin-C	0.0010	50	1,050	643	0.61	12.9	26.0	56.83
	0.0040	10	209	232	1.11	23.2	26.0	184.28
4,4′-Diamino-2,2′-stilbenedisulfonic acid								
	500	50	1,051	427	0.40	8.5	26.0	4.05
	1,667	50	1,048	391	0.37	7.8	26.0	−4.45
	5,000	50	1,051	475	0.45	9.5	31.0[d]	15.75
								P=0.046
+S9								
Trial 1								
Summary: Negative								
Distilled water		50	1,050	431	0.41	8.6	26.0	
		50	1,044	421	0.40	8.4	31.0[d]	
Cyclophosphamide	0.1250	50	1,047	540	0.51	10.8	26.0	27.90
	0.5000	10	211	200	0.94	20.0	26.0	135.06
4,4′-Diamino-2,2′-stilbenedisulfonic acid								
	500	50	1,049	405	0.38	8.1	26.0	−4.26
	1,667	50	1,049	448	0.42	9.0	26.0	5.91
	5,000	50	1,050	457	0.43	9.1	31.0[d]	7.93
								P=0.060

[a] SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. The data from the study performed at Bioassay Systems Corporation is published in Loveday *et al.* (1990).
[b] Percent increase in SCEs/chromosome of culture exposed to 4,4′-diamino-2,2′-stilbenedisulfonic acid relative to those of culture exposed to solvent.
[c] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose
[d] Because a chemical-induced cell cycle delay was seen, incubation time was lengthened five hours to ensure a sufficient number of scorable cells.

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 4,4′-Diamino-2,2′-stilbenedisulfonic Acid[a]

-S9					+S9				
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
Study performed at Bioassay Systems Corporation									
Trial 1 – Harvest time: 10.0 hours Summary: Negative					**Trial 1** – Harvest time: 12.0 hours Summary: Negative				
Medium					Medium				
	200	1	0.01	0.5		200	5	0.03	2.0
Mitomycin-C					Cyclophosphamide				
1.0	200	48	0.24	19.0	50.0	50	43	0.86	36.0
5.0	50	16	0.32	28.0					
4,4′-Diamino-2,2′-stilbenedisulfonic acid					4,4′-Diamino-2,2′-stilbenedisulfonic acid				
101	200	6	0.03	3.0	101	200	5	0.03	1.5
303	200	4	0.02	1.5	303	200	3	0.02	1.5
1,010	200	4	0.02	2.0	1,010	200	0	0.00	0.0
				P=0.232[b]					P=0.957
Study performed at Sitek Research Laboratories									
Trial 1 – Harvest time: 15.0 hours[c] Summary: Negative					**Trial 1** – Harvest time: 12.5 hours Summary: Negative				
Distilled water					Distilled water				
	200	0	0.00	0.0		200	2	0.01	1.0
Mitomycin-C					Cyclophosphamide				
0.4	25	25	1.00	64.0	20.0	25	23	0.92	56.0
4,4′-Diamino-2,2′-stilbenedisulfonic acid					4,4′-Diamino-2,2′-stilbenedisulfonic acid				
1,081	200	1	0.01	0.5	1,081	200	13	0.07	2.0
2,325	200	0	0.00	0.0	2,325	200	3	0.02	1.5
5,000	200	1	0.01	0.5	5,000	200	1	0.01	0.5
				P=0.263					P=0.715

[a] Abs = aberrations. The data from the study performed at Bioassay Systems Corporation is published in Loveday *et al.* (1990).
[b] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose
[c] Because of chemical-induced cell cycle delay, incubation time prior to additon of Colcemid was lengthened to provide sufficient metaphases at harvest.

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

TABLE F1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 200

TABLE F2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 201

TABLE F3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluations in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 202

TABLE F4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 203

TABLE F5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 204

TABLE F6 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluations in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 205

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm	100,000 ppm
Male						
n	5	5	5	5	5	5
Necropsy body wt	211 ± 5	217 ± 6	211 ± 7	216 ± 4	199 ± 3	185 ± 4**
Brain						
Absolute	1.67 ± 0.02	1.69 ± 0.04	1.73 ± 0.05	1.73 ± 0.02	1.69 ± 0.04	1.69 ± 0.06
Relative	7.92 ± 0.16	7.82 ± 0.26	8.20 ± 0.10	8.01 ± 0.19	8.50 ± 0.24	9.15 ± 0.26**
Heart						
Absolute	0.78 ± 0.01	1.06 ± 0.17	0.87 ± 0.03	0.83 ± 0.03	0.77 ± 0.02	0.68 ± 0.01
Relative	3.72 ± 0.09	4.95 ± 0.97	4.13 ± 0.13	3.82 ± 0.09	3.90 ± 0.13	3.66 ± 0.07
R. Kidney						
Absolute	1.02 ± 0.03	1.08 ± 0.03	1.02 ± 0.07	1.05 ± 0.03	1.02 ± 0.02	0.95 ± 0.03
Relative	4.82 ± 0.11	4.96 ± 0.06	4.82 ± 0.24	4.87 ± 0.15	5.15 ± 0.10	5.14 ± 0.11
Liver						
Absolute	11.01 ± 0.51	10.82 ± 0.40	11.65 ± 0.66	11.62 ± 0.26	11.67 ± 0.10	11.16 ± 0.43
Relative	52.2 ± 1.5	49.7 ± 1.1	55.2 ± 2.4	53.7 ± 1.2	58.8 ± 0.9**	60.4 ± 1.4**
Lungs						
Absolute	1.19 ± 0.08	1.40 ± 0.17	1.54 ± 0.19	1.50 ± 0.12	1.11 ± 0.04	1.09 ± 0.06
Relative	5.67 ± 0.44	6.45 ± 0.81	7.41 ± 1.11	6.93 ± 0.56	5.59 ± 0.23	5.90 ± 0.31
Thymus						
Absolute	0.45 ± 0.01	0.49 ± 0.03	0.42 ± 0.03	0.45 ± 0.03	0.42 ± 0.02	0.39 ± 0.03
Relative	2.14 ± 0.09	2.26 ± 0.16	1.99 ± 0.08	2.08 ± 0.14	2.13 ± 0.13	2.13 ± 0.14
Female						
n	5	5	5	5	5	5
Necropsy body wt	149 ± 3	149 ± 5	147 ± 5	153 ± 5	145 ± 2	145 ± 3
Brain						
Absolute	1.58 ± 0.03	1.60 ± 0.05	1.61 ± 0.02	1.65 ± 0.02	1.61 ± 0.03	1.62 ± 0.03
Relative	10.6 ± 0.3	10.8 ± 0.2	11.0 ± 0.3	10.8 ± 0.2	11.1 ± 0.2	11.2 ± 0.2
Heart						
Absolute	0.62 ± 0.02	0.63 ± 0.04	0.62 ± 0.02	0.64 ± 0.03	0.60 ± 0.02	0.57 ± 0.01
Relative	4.18 ± 0.13	4.22 ± 0.22	4.23 ± 0.11	4.17 ± 0.11	4.13 ± 0.10	3.97 ± 0.14
R. Kidney						
Absolute	0.74 ± 0.01	0.75 ± 0.03	0.71 ± 0.02	0.77 ± 0.05	0.74 ± 0.04	0.77 ± 0.03
Relative	4.98 ± 0.09	5.05 ± 0.15	4.83 ± 0.15	5.02 ± 0.16	5.11 ± 0.23	5.31 ± 0.11
Liver						
Absolute	7.21 ± 0.15	7.20 ± 0.24	7.30 ± 0.31	7.73 ± 0.24	7.18 ± 0.18	8.02 ± 0.34*
Relative	48.6 ± 1.6	48.5 ± 0.8	49.7 ± 1.1	50.7 ± 1.4	49.6 ± 1.0	55.3 ± 1.4**
Lungs						
Absolute	0.89 ± 0.03	0.99 ± 0.07	0.96 ± 0.05	1.28 ± 0.23	1.02 ± 0.04	0.89 ± 0.03
Relative	6.01 ± 0.21	6.68 ± 0.38	6.53 ± 0.17	8.42 ± 1.56	7.05 ± 0.33	6.13 ± 0.14
Thymus						
Absolute	0.36 ± 0.02	0.36 ± 0.02	0.37 ± 0.01	0.36 ± 0.02	0.36 ± 0.01	0.36 ± 0.03
Relative	2.41 ± 0.14	2.39 ± 0.08	2.50 ± 0.06	2.37 ± 0.11	2.48 ± 0.07	2.45 ± 0.19

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

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm	100,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	361 ± 5	359 ± 3	347 ± 3	353 ± 4	326 ± 7**	255 ± 4**
Brain						
Absolute	1.87 ± 0.03	1.88 ± 0.02	1.83 ± 0.03	1.81 ± 0.04	1.88 ± 0.04	1.83 ± 0.02
Relative	5.18 ± 0.10	5.25 ± 0.09	5.29 ± 0.09	5.13 ± 0.10	5.80 ± 0.10**	7.20 ± 0.11**
Heart						
Absolute	1.21 ± 0.02	1.16 ± 0.02	1.15 ± 0.02	1.19 ± 0.02	1.13 ± 0.02*	1.02 ± 0.02**
Relative	3.35 ± 0.06	3.25 ± 0.06	3.32 ± 0.05	3.37 ± 0.06	3.48 ± 0.05	3.99 ± 0.06**
R. Kidney						
Absolute	1.38 ± 0.03	1.36 ± 0.03	1.31 ± 0.02	1.40 ± 0.03	1.35 ± 0.04	1.24 ± 0.02**
Relative	3.82 ± 0.09	3.80 ± 0.07	3.79 ± 0.05	3.96 ± 0.07	4.15 ± 0.09**	4.86 ± 0.05**
Liver						
Absolute	12.67 ± 0.32	12.31 ± 0.30	11.68 ± 0.29	13.00 ± 0.27	12.24 ± 0.30	9.94 ± 0.29**
Relative	35.1 ± 0.6	34.3 ± 0.6	33.7 ± 0.8	36.8 ± 0.4	37.6 ± 0.5**	38.9 ± 0.9**
Lungs						
Absolute	1.57 ± 0.02	1.56 ± 0.02	1.71 ± 0.12	1.65 ± 0.06	1.50 ± 0.04	1.33 ± 0.03**
Relative	4.37 ± 0.08	4.34 ± 0.06	4.92 ± 0.33	4.68 ± 0.17	4.60 ± 0.10	5.20 ± 0.11**
R. Testis						
Absolute	1.53 ± 0.02	1.54 ± 0.03	1.52 ± 0.02	1.56 ± 0.03	1.50 ± 0.02	1.42 ± 0.03**
Relative	4.25 ± 0.07	4.29 ± 0.07	4.39 ± 0.06	4.44 ± 0.09	4.63 ± 0.09**	5.56 ± 0.13**
Thymus						
Absolute	0.31 ± 0.02	0.32 ± 0.01	0.28 ± 0.01	0.30 ± 0.01	0.29 ± 0.01[b]	0.22 ± 0.01**
Relative	0.85 ± 0.04	0.88 ± 0.03	0.81 ± 0.04	0.85 ± 0.03	0.90 ± 0.04[b]	0.87 ± 0.02
Female						
n	10	10	10	10	10	9
Necropsy body wt	196 ± 3	194 ± 3	194 ± 3	189 ± 2	187 ± 4	174 ± 6**
Brain						
Absolute	1.68 ± 0.02	1.73 ± 0.02	1.75 ± 0.02	1.75 ± 0.01	1.76 ± 0.03	1.67 ± 0.03
Relative	8.61 ± 0.13	8.93 ± 0.19	9.04 ± 0.13	9.28 ± 0.12**	9.43 ± 0.22**	9.66 ± 0.23**
Heart						
Absolute	0.77 ± 0.02	0.75 ± 0.02	0.78 ± 0.01	0.76 ± 0.02	0.76 ± 0.02	0.74 ± 0.02
Relative	3.93 ± 0.05	3.87 ± 0.07	4.00 ± 0.06	4.00 ± 0.10	4.06 ± 0.09	4.25 ± 0.06**
R. Kidney						
Absolute	0.77 ± 0.02	0.77 ± 0.01	0.78 ± 0.01	0.78 ± 0.02	0.82 ± 0.02	0.80 ± 0.02
Relative	3.93 ± 0.09	3.97 ± 0.05	4.03 ± 0.07	4.14 ± 0.08	4.38 ± 0.07**	4.59 ± 0.12**
Liver						
Absolute	6.45 ± 0.19	6.44 ± 0.11	6.63 ± 0.08	6.50 ± 0.21	6.09 ± 0.14	6.58 ± 0.21
Relative	33.0 ± 0.9	33.2 ± 0.5	34.2 ± 0.4	34.3 ± 0.9	32.7 ± 0.7	37.9 ± 0.8**
Lungs						
Absolute	1.13 ± 0.03	1.14 ± 0.02	1.14 ± 0.02	1.16 ± 0.04	1.15 ± 0.03	1.05 ± 0.03
Relative	5.76 ± 0.14	5.88 ± 0.13	5.85 ± 0.11	6.11 ± 0.14	6.17 ± 0.18	6.06 ± 0.13
Thymus						
Absolute	0.27 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.23 ± 0.01**
Relative	1.38 ± 0.03	1.35 ± 0.04	1.31 ± 0.04	1.35 ± 0.05	1.30 ± 0.05	1.30 ± 0.05

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

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluations in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	12,500 ppm	25,000 ppm
Male			
n	10	10	10
Necropsy body wt	473 ± 14	459 ± 9	436 ± 9*
Brain			
Absolute	2.07 ± 0.03	2.01 ± 0.02	2.03 ± 0.03
Relative	4.40 ± 0.13	4.39 ± 0.11	4.68 ± 0.09
R. Kidney			
Absolute	1.85 ± 0.04	1.90 ± 0.06	1.81 ± 0.05
Relative	3.92 ± 0.11	4.15 ± 0.10	4.17 ± 0.14
Liver			
Absolute	14.01 ± 0.53	15.00 ± 0.53	13.62 ± 0.31
Relative	29.7 ± 0.8	32.7 ± 1.2	31.4 ± 0.9
Female			
n	10	10	10
Necropsy body wt	300 ± 6	283 ± 4	283 ± 6
Brain			
Absolute	1.86 ± 0.03	1.81 ± 0.03	1.85 ± 0.03
Relative	6.22 ± 0.09	6.39 ± 0.09	6.57 ± 0.15
R. Kidney			
Absolute	1.21 ± 0.05	1.16 ± 0.05	1.16 ± 0.05
Relative	4.02 ± 0.12	4.09 ± 0.17	4.10 ± 0.16
Liver			
Absolute	8.72 ± 0.26	8.31 ± 0.27	8.37 ± 0.24
Relative	29.1 ± 0.8	29.4 ± 1.0	29.6 ± 0.6

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

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm	100,000 ppm
Male						
n	5	5	5	5	5	5
Necropsy body wt	24.2 ± 0.7	24.2 ± 0.7	24.4 ± 0.5	23.4 ± 0.2	23.8 ± 0.7	22.2 ± 0.9*
Brain						
Absolute	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.00	0.43 ± 0.01	0.41 ± 0.02	0.44 ± 0.04
Relative	17.8 ± 0.8	18.0 ± 0.8	17.6 ± 0.4	18.4 ± 0.3	17.3 ± 1.2	19.7 ± 1.8
Heart						
Absolute	0.13 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.01
Relative	5.49 ± 0.36	5.52 ± 0.28	6.02 ± 0.34	5.39 ± 0.33	5.37 ± 0.13	5.10 ± 0.15
R. Kidney						
Absolute	0.23 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.24 ± 0.02	0.20 ± 0.01*
Relative	9.64 ± 0.19	9.97 ± 0.17	9.19 ± 0.44	9.42 ± 0.29	9.92 ± 0.48	8.87 ± 0.18
Liver						
Absolute	1.40 ± 0.06	1.37 ± 0.04	1.36 ± 0.03	1.32 ± 0.02	1.43 ± 0.05	1.49 ± 0.04
Relative	57.9 ± 1.4	56.7 ± 2.2	55.6 ± 0.7	56.6 ± 1.0	60.1 ± 1.0	67.6 ± 3.7**
Lungs						
Absolute	0.18 ± 0.01	0.20 ± 0.01	0.25 ± 0.02**	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01
Relative	7.43 ± 0.36	8.14 ± 0.43	10.32 ± 0.93**	7.64 ± 0.36	7.43 ± 0.41	7.92 ± 0.59
Thymus[b]						
Absolute	47.60 ± 4.13	51.75 ± 2.29[c]	57.20 ± 6.26	45.60 ± 4.06	47.20 ± 2.85	27.00 ± 4.64**
Relative	1.96 ± 0.14	2.14 ± 0.12	2.35 ± 0.27	1.95 ± 0.17	1.99 ± 0.14	1.24 ± 0.24*
Female						
n	5	5	5	5	5	5
Necropsy body wt	21.0 ± 0.6	20.2 ± 0.6	20.0 ± 0.8	20.8 ± 0.6	20.0 ± 0.7	19.4 ± 0.6
Brain						
Absolute	0.46 ± 0.01	0.44 ± 0.00	0.45 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.44 ± 0.02
Relative	21.9 ± 1.0	21.6 ± 0.6	22.4 ± 0.8	21.1 ± 0.7	21.9 ± 1.2	22.5 ± 0.8
Heart						
Absolute	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.03	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Relative	5.62 ± 0.18	5.96 ± 0.24	7.58 ± 1.58	5.65 ± 0.29	5.87 ± 0.54	5.61 ± 0.29
R. Kidney						
Absolute	0.18 ± 0.00	0.17 ± 0.00	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
Relative	8.36 ± 0.14	8.42 ± 0.15	8.45 ± 0.22	8.30 ± 0.54	8.44 ± 0.25	8.33 ± 0.26
Liver						
Absolute	1.12 ± 0.06	1.07 ± 0.03	1.09 ± 0.03	1.10 ± 0.05	1.11 ± 0.04	1.18 ± 0.04
Relative	53.5 ± 2.8	53.3 ± 1.2	54.6 ± 1.7	52.8 ± 2.2	55.3 ± 0.6	60.9 ± 1.8*
Lungs						
Absolute	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.16 ± 0.02
Relative	8.09 ± 0.59	8.99 ± 0.19	8.82 ± 0.48	8.07 ± 0.19	8.82 ± 0.28	8.17 ± 0.77
Thymus[b]						
Absolute	56.00 ± 1.05	53.40 ± 4.23	56.20 ± 3.43	56.20 ± 2.13	54.20 ± 4.35	23.80 ± 3.32**
Relative	2.68 ± 0.13	2.64 ± 0.19	2.81 ± 0.10	2.70 ± 0.08	2.73 ± 0.26	1.22 ± 0.14**

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

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm	100,000 ppm
Male						
n	10	10	10	10	10	4
Necropsy body wt	26.0 ± 0.6	26.2 ± 0.8	25.7 ± 0.6	25.0 ± 0.5	23.6 ± 0.5**	17.0 ± 0.9**
Brain						
Absolute	0.41 ± 0.01	0.42 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.42 ± 0.01	0.39 ± 0.01
Relative	16.0 ± 0.3	16.0 ± 0.4	17.1 ± 0.5	17.1 ± 0.4	17.9 ± 0.3**	23.0 ± 1.0**
Heart						
Absolute	0.157 ± 0.006	0.159 ± 0.007	0.158 ± 0.007	0.151 ± 0.007	0.139 ± 0.006	0.128 ± 0.005*
Relative	6.05 ± 0.14	6.06 ± 0.17	6.16 ± 0.23	6.04 ± 0.20	5.89 ± 0.17	7.61 ± 0.52**
R. Kidney						
Absolute	0.23 ± 0.00	0.24 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.21 ± 0.01*	0.16 ± 0.01**
Relative	8.95 ± 0.17	9.10 ± 0.22	9.12 ± 0.14	9.24 ± 0.19	8.89 ± 0.18	9.45 ± 0.23
Liver						
Absolute	1.09 ± 0.04	1.07 ± 0.04	1.02 ± 0.03	0.99 ± 0.03*	0.91 ± 0.03**	0.83 ± 0.04**
Relative	41.8 ± 1.2	40.8 ± 1.1	39.8 ± 0.7	39.3 ± 0.5	38.4 ± 0.5*	49.1 ± 2.5**
Lungs						
Absolute	0.21 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.15 ± 0.01**
Relative	7.95 ± 0.30	7.91 ± 0.27	7.80 ± 0.24	8.10 ± 0.20	7.96 ± 0.25	8.94 ± 0.56
R. Testis						
Absolute	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.00	0.07 ± 0.01**
Relative	4.49 ± 0.11	4.42 ± 0.12	4.49 ± 0.18	4.63 ± 0.21	4.54 ± 0.05	4.29 ± 0.42
Thymus[b]						
Absolute	28.40 ± 3.10	31.80 ± 2.64	31.44 ± 2.94[c]	28.00 ± 1.88	35.90 ± 3.26	16.50 ± 3.86
Relative	1.09 ± 0.11	1.21 ± 0.09	1.24 ± 0.12[c]	1.12 ± 0.06	1.51 ± 0.12*	0.95 ± 0.18
Female						
n	10	10	10	10	10	9
Necropsy body wt	21.4 ± 0.4	20.4 ± 0.3	20.5 ± 0.3	20.6 ± 0.7	19.9 ± 0.4*	15.7 ± 0.3**
Brain						
Absolute	0.45 ± 0.01	0.47 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.39 ± 0.01**
Relative	20.9 ± 0.3	22.9 ± 0.5	21.6 ± 0.4	21.2 ± 0.6	21.9 ± 0.4	24.8 ± 0.7**
Heart						
Absolute	0.132 ± 0.004	0.130 ± 0.004	0.122 ± 0.002	0.125 ± 0.004	0.116 ± 0.004**	0.118 ± 0.005**
Relative	6.17 ± 0.15	6.38 ± 0.20	5.95 ± 0.09	6.12 ± 0.24	5.83 ± 0.14	7.53 ± 0.24**
R. Kidney						
Absolute	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.15 ± 0.00
Relative	7.58 ± 0.29	8.48 ± 0.19	7.81 ± 0.17	8.20 ± 0.18	8.06 ± 0.20	9.27 ± 0.13**
Liver						
Absolute	0.87 ± 0.02	0.84 ± 0.02	0.86 ± 0.02	0.85 ± 0.04	0.83 ± 0.03	0.76 ± 0.02**
Relative	40.9 ± 0.5	41.0 ± 0.6	42.0 ± 0.8	41.0 ± 1.3	41.7 ± 1.2	48.8 ± 0.9**
Lungs						
Absolute	0.18 ± 0.01	0.21 ± 0.01	0.19 ± 0.01[c]	0.18 ± 0.01	0.18 ± 0.01	0.14 ± 0.01**
Relative	8.64 ± 0.22	10.13 ± 0.50	9.48 ± 0.38[c]	9.00 ± 0.44	9.04 ± 0.44	9.03 ± 0.55
Thymus[b]						
Absolute	37.20 ± 1.85	38.70 ± 2.02	39.60 ± 3.20	39.30 ± 2.56	44.10 ± 2.06	15.20 ± 4.19**[d]
Relative	1.74 ± 0.08	1.91 ± 0.11	1.95 ± 0.18	1.90 ± 0.11	2.21 ± 0.09*	0.96 ± 0.28**[d]

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams unless otherwise noted; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] Thymus weights are given in milligrams.
[c] n=9
[d] n=5

TABLE F6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluations in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

	0 ppm	6,250 ppm	12,500 ppm
Male			
n	10	10	10
Necropsy body wt	34.2 ± 2.0	36.4 ± 1.0	33.7 ± 1.1
Brain			
Absolute	0.46 ± 0.01	0.49 ± 0.02	0.47 ± 0.02
Relative	13.8 ± 0.9	13.7 ± 0.7	14.1 ± 0.9
R. Kidney			
Absolute	0.37 ± 0.05	0.42 ± 0.04	0.36 ± 0.02
Relative	11.1 ± 1.6	11.6 ± 1.4	10.6 ± 0.3
Liver			
Absolute	1.24 ± 0.08	1.46 ± 0.05	1.36 ± 0.05
Relative	36.2 ± 1.0	40.2 ± 1.4	40.8 ± 2.1
Female			
n	10	10	10
Necropsy body wt	31.6 ± 1.6	30.0 ± 2.0	28.9 ± 1.1
Brain			
Absolute	0.49 ± 0.01	0.47 ± 0.01	0.48 ± 0.02
Relative	15.7 ± 0.7	16.3 ± 1.1	17.2 ± 1.3
R. Kidney			
Absolute	0.22 ± 0.01	0.25 ± 0.04	0.23 ± 0.03
Relative	6.99 ± 0.38	8.50 ± 1.45	8.14 ± 1.12
Liver			
Absolute	1.25 ± 0.07	1.22 ± 0.09	1.15 ± 0.04
Relative	39.7 ± 2.1	41.9 ± 3.6	40.2 ± 0.9

[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error). Differences from the control group are not significant by Williams' or Dunnett's test.

APPENDIX G
HEMATOLOGY AND CLINICAL CHEMISTRY RESULTS

TABLE G1 Clinical Chemistry Data for Rats in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 208

TABLE G2 Hematology and Clinical Chemistry Data for Rats at the 15-Month Interim Evaluations of the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 209

TABLE G3 Clinical Chemistry Data for Mice in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 210

TABLE G4 Hematology and Clinical Chemistry Data for Mice at the 15-Month Interim Evaluations of the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 211

TABLE G1
Clinical Chemistry Data for Rats in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

Analysis	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm	100,000 ppm
Male						
n	10	10	10	10	10	10
Serum glucose (mg/dL)	106 ± 4	104 ± 2	109 ± 4	106 ± 3	101 ± 3	99 ± 1
Total protein (g/dL)	7.0 ± 0.1	6.7 ± 0.1*	6.7 ± 0.1*	6.8 ± 0.1*	6.9 ± 0.1	6.6 ± 0.1**
Albumin (g/dL)	3.7 ± 0.0	3.6 ± 0.0*	3.6 ± 0.0*	3.6 ± 0.0*	3.6 ± 0.0	3.4 ± 0.0**
A/G ratio	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.1 ± 0.0	1.1 ± 0.0**
Alkaline phosphatase (IU/L)	75 ± 2	70 ± 3	71 ± 3	69 ± 1	71 ± 1	81 ± 2
ALT (IU/L)	44 ± 1	41 ± 1	43 ± 1	39 ± 1*	33 ± 1**	33 ± 1**
AST (IU/L)	72 ± 3	70 ± 2	75 ± 3	74 ± 5	73 ± 4	82 ± 3*
LDH (IU/L)	523 ± 57	512 ± 29	489 ± 37	479 ± 36	569 ± 48	613 ± 50
Female						
n	10	10	10	10	10	9
Serum glucose (mg/dL)	95 ± 3	96 ± 4	101 ± 4	98 ± 3	93 ± 3	93 ± 5
Total protein (g/dL)	6.8 ± 0.2	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.5 ± 0.1	5.9 ± 0.1**
Albumin (g/dL)	3.7 ± 0.1	3.7 ± 0.1	3.8 ± 0.0	3.7 ± 0.1	3.7 ± 0.1	3.2 ± 0.1**
A/G ratio	1.2 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.1 ± 0.0
Alkaline phosphatase (IU/L)	55 ± 2	55 ± 2	54 ± 2	52 ± 2	58 ± 2	62 ± 3*
ALT (IU/L)	36 ± 1	38 ± 2	36 ± 1	39 ± 1	34 ± 1	33 ± 1
AST (IU/L)	68 ± 2	73 ± 4	72 ± 4	74 ± 3	71 ± 3	76 ± 3
LDH (IU/L)	363 ± 30	398 ± 38	372 ± 39	386 ± 42	402 ± 46	458 ± 41

* 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. A/G ratio=albumin/giobulin ratio; ALT=alanine aminotransferase; AST=aspartate aminotransferase; LDH=lactate dehydrogenase

TABLE G2
Hematology and Clinical Chemistry Data for Rats at the 15-Month Interim Evaluations in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

Analysis	0 ppm	12,500 ppm	25,000 ppm
Male			
n	10	10	10
Hematology			
Hematocrit (%)	52.5 ± 1.0	53.4 ± 0.5	53.8 ± 0.6
Hemoglobin (g/dL)	15.3 ± 0.3	15.4 ± 0.2	15.5 ± 0.2
Erythrocytes ($10^6/\mu L$)	8.06 ± 0.11	8.11 ± 0.07	8.16 ± 0.09
Mean cell volume (μ^3)	65.2 ± 1.0	65.7 ± 0.2	66.0 ± 0.3
Mean cell hemoglobin (pg)	19.0 ± 0.3	19.1 ± 0.1	19.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	29.1 ± 0.1	28.9 ± 0.1	28.9 ± 0.1
Leukocytes ($10^3/\mu L$)	9.10 ± 1.07	7.86 ± 0.27	7.62 ± 0.53
Segmented neutrophils ($10^3/\mu L$)	4.10 ± 0.72	3.24 ± 0.22	3.26 ± 0.34
Lymphocytes ($10^3/\mu L$)	4.63 ± 0.39	4.38 ± 0.30	4.10 ± 0.26
Monocytes ($10^3/\mu L$)	0.09 ± 0.02	0.07 ± 0.02	0.08 ± 0.02
Eosinophils ($10^3/\mu L$)	0.25 ± 0.06	0.20 ± 0.04	0.18 ± 0.04
Clinical Chemistry			
Blood urea nitrogen (mg/dL)	13.7 ± 0.5	14.8 ± 0.3	14.0 ± 0.4
Alkaline phosphatase (IU/L)	56 ± 5	56 ± 2	54 ± 1
ALT (IU/L)	49 ± 4	51 ± 3	42 ± 2
AST (IU/L)	73 ± 9	74 ± 4	64 ± 3
SDH (IU/L)	11 ± 0	11 ± 1	10 ± 0
Female			
n	10	10	10
Hematology			
Hematocrit (%)	53.0 ± 0.5	52.7 ± 0.5	48.7 ± 3.9
Hemoglobin (g/dL)	15.6 ± 0.2	15.5 ± 0.1	14.4 ± 1.1
Erythrocytes ($10^6/\mu L$)	7.55 ± 0.08	7.50 ± 0.08	6.91 ± 0.56
Mean cell volume (μ^3)	70.1 ± 0.2	70.1 ± 0.1	70.5 ± 0.2
Mean cell hemoglobin (pg)	20.7 ± 0.1	20.6 ± 0.1	21.0 ± 0.2
Mean cell hemoglobin concentration (g/dL)	29.5 ± 0.1	29.4 ± 0.1	29.7 ± 0.2
Leukocytes ($10^3/\mu L$)	4.07 ± 0.20	3.54 ± 0.20	4.95 ± 0.71
Segmented neutrophils ($10^3/\mu L$)	1.49 ± 0.22	1.18 ± 0.09	2.27 ± 0.51
Lymphocytes ($10^3/\mu L$)	2.40 ± 0.15	2.26 ± 0.16	2.57 ± 0.24
Monocytes ($10^3/\mu L$)	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02
Eosinophils ($10^3/\mu L$)	0.11 ± 0.02	0.07 ± 0.02	0.09 ± 0.02
Clinical Chemistry			
Blood urea nitrogen (mg/dL)	12.5 ± 0.3	13.5 ± 0.6	14.7 ± 1.0
Alkaline phosphatase (IU/L)	38 ± 1	34 ± 2	35 ± 4
ALT (IU/L)	29 ± 1	28 ± 1	27 ± 1
AST (IU/L)	50 ± 2	50 ± 2	49 ± 2
SDH (IU/L)	8 ± 0	8 ± 0	8 ± 0

[a] Mean ± standard error. Differences from the control group are not significant by Dunn's or Shirley's test. ALT=alanine aminotransferase; AST=aspartate aminotransferase; SDH=sorbitol dehydrogenase

TABLE G3
Clinical Chemistry Data for Mice in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt[a]

Analysis	0 ppm	6,250 ppm	12,500 ppm	25,000 ppm	50,000 ppm	100,000 ppm
Male						
n	10	10	10	10	10	4
Serum glucose (mg/dL)	99 ± 4	102 ± 6	111 ± 9	113 ± 4	116 ± 7	95 ± 8
Total protein (g/dL)	5.9 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.1 ± 0.1**
Albumin (g/dL)	3.2 ± 0.1	3.3 ± 0.1	3.2 ± 0.0	3.2 ± 0.1	3.2 ± 0.1	2.7 ± 0.1**
A/G ratio	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.1	1.2 ± 0.1
Alkaline phosphatase (IU/L)	40 ± 2	45 ± 2	42 ± 1	45 ± 2	49 ± 1**	22 ± 1
ALT (IU/L)	148 ± 21	156 ± 22	141 ± 30	143 ± 23	120 ± 12	68 ± 5*
AST (IU/L)	129 ± 7	145 ± 12	142 ± 12	126 ± 15	132 ± 11	103 ± 11
LDH (IU/L)	592 ± 30	588 ± 48	543 ± 50	550 ± 51	530 ± 29	573 ± 56
Female						
n	10	10	10	10	10	9
Serum glucose (mg/dL)	85 ± 7	78 ± 7	94 ± 10	78 ± 5	112 ± 8*	99 ± 9
Total protein (g/dL)	5.9 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	6.0 ± 0.2	4.7 ± 0.1**
Albumin (g/dL)	3.5 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	3.4 ± 0.1	3.5 ± 0.1	2.6 ± 0.1**
A/G ratio	1.5 ± 0.0	1.5 ± 0.1	1.5 ± 0.0	1.4 ± 0.0	1.4 ± 0.1	1.2 ± 0.1**
Alkaline phosphatase (IU/L)	77 ± 2	75 ± 3	69 ± 2*	76 ± 3	78 ± 3	21 ± 1**
ALT (IU/L)	81 ± 12	87 ± 32	77 ± 16	85 ± 13	97 ± 13	121 ± 20
AST (IU/L)	115 ± 10	141 ± 37	94 ± 7	118 ± 7	130 ± 14	119 ± 10
LDH (IU/L)	418 ± 27	436 ± 27	396 ± 33	431 ± 23	472 ± 38	551 ± 54

* 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. A/G ratio=albumin/globulin ratio; ALT=alanine aminotransferase; AST=aspartate aminotransferase; LDH=lactate dehydrogenase

TABLE G4
Hematology and Clinical Chemistry Data for Mice at the 15-Month Interim Evaluations in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-Stilbenesulfonic Acid, Disodium Salt[a]

Analysis	0 ppm	6,250 ppm	12,500 ppm
Male			
n	10	10	10
Hematology			
Hematocrit (%)	38.8 ± 1.5	39.7 ± 0.5	39.6 ± 0.9
Hemoglobin (g/dL)	15.9 ± 0.3	15.7 ± 0.3	15.7 ± 0.4
Erythrocytes ($10^6/\mu L$)	7.91 ± 0.29	8.08 ± 0.12	8.16 ± 0.23
Mean cell volume (μ^3)	49.1 ± 0.2	49.1 ± 0.2	48.4 ± 0.3
Mean cell hemoglobin (pg)	20.3 ± 0.7	19.5 ± 0.2	19.2 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	41.4 ± 1.4	39.6 ± 0.3	39.7 ± 0.3
Leukocytes ($10^3/\mu L$)	4.18 ± 0.46	5.84 ± 0.69	5.01 ± 0.41
Segmented neutrophils ($10^3/\mu L$)	0.73 ± 0.07	1.07 ± 0.14	0.85 ± 0.11
Lymphocytes ($10^3/\mu L$)	3.36 ± 0.41	4.51 ± 0.56	4.01 ± 0.37
Monocytes ($10^3/\mu L$)	0.05 ± 0.02	0.12 ± 0.04	0.04 ± 0.02
Eosinophils ($10^3/\mu L$)	0.06 ± 0.02	0.12 ± 0.03	0.10 ± 0.03
Clinical Chemistry			
Blood urea nitrogen (mg/dL)	22.9 ± 2.2[b]	23.3 ± 1.5	22.1 ± 1.1[b]
Alkaline phosphatase (IU/L)	38 ± 2	39 ± 1	39 ± 1[b]
ALT (IU/L)	50 ± 5	46 ± 5	49 ± 10[b]
AST (IU/L)	75 ± 11	90 ± 14	71 ± 7[b]
SDH (IU/L)	43 ± 6[b]	36 ± 2	37 ± 1[b]
Female			
n	10	10	10
Hematology			
Hematocrit (%)	39.8 ± 0.6	39.3 ± 0.8	39.7 ± 0.7
Hemoglobin (g/dL)	15.6 ± 0.2	15.6 ± 0.3	15.8 ± 0.3
Erythrocytes ($10^6/\mu L$)	8.09 ± 0.11	8.05 ± 0.14	8.02 ± 0.14
Mean cell volume (μ^3)	49.0 ± 0.3	48.9 ± 0.3	49.4 ± 0.2
Mean cell hemoglobin (pg)	19.3 ± 0.1	19.3 ± 0.1	19.7 ± 0.2
Mean cell hemoglobin concentration (g/dL)	39.3 ± 0.2	39.6 ± 0.2	39.7 ± 0.4
Leukocytes ($10^3/\mu L$)	4.49 ± 0.40	4.51 ± 0.61	4.50 ± 0.25
Segmented neutrophils ($10^3/\mu L$)	0.59 ± 0.07	0.64 ± 0.08	0.71 ± 0.08
Lymphocytes ($10^3/\mu L$)	3.76 ± 0.34	3.69 ± 0.49	3.66 ± 0.23
Monocytes ($10^3/\mu L$)	0.08 ± 0.02	0.07 ± 0.03	0.09 ± 0.02
Eosinophils ($10^3/\mu L$)	0.03 ± 0.02	0.08 ± 0.03	0.04 ± 0.02
Clinical Chemistry			
Blood urea nitrogen (mg/dL)	15.2 ± 0.5	17.5 ± 1.0*	17.2 ± 0.7*
Alkaline phosphatase (IU/L)	70 ± 5	74 ± 5	64 ± 3
ALT (IU/L)	44 ± 6	45 ± 5	41 ± 3
AST (IU/L)	90 ± 7	94 ± 9	90 ± 9
SDH (IU/L)	29 ± 1	31 ± 2	29 ± 1

* 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. ALT=alanine aminotransferase; AST=aspartate aminotransferase; SDH=sorbitol dehydrogenase
[b] n=9

APPENDIX H
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, was obtained from Ciba-Geigy Corporation in one lot (SW-81605), which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (MRI; Kansas City, MO). MRI reports on analyses performed in support of the 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a yellow microcrystalline powder, was identified as 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, by infrared, ultraviolet/visible, and nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, as shown in Figures H1 and H2 (*Sadtler Standard Spectra*).

Initially, the bulk chemical was divided into three subbatches. The relative purity of the three subbatches was determined by high-performance liquid chromatography (HPLC) with a μBondapak C_{18} column in a mixture of two solvents: A) 0.005 M tetrabutylammonium hydroxide in water, with pH adjusted to 7.4 with 2% phosphoric acid, and B) 0.005 M tetrabutylammonium hydroxide in methanol, with an equal volume of phosphoric acid as added in solvent A. The solvent ratio was 65:35 (A:B), premixed, and the flow rate was 2.0 mL/minute. Ultraviolet detection was at 254 nm. The three subbatches were determined to have the same purity.

The purity of the bulk chemical was determined by elemental analysis, weight loss on drying, thin-layer chromatography (TLC), and HPLC. TLC was performed on silica gel 60 F-254 plates with two solvent systems: A) phenol:water:acetic acid (75 g:20 mL:10 mL), and B) *n*-butanol:pyridine:water (33:33:33). Visualization was accomplished with visible light, long-wave (366 nm) ultraviolet light, and a spray of 4-dimethylaminobenzaldehyde (1 g in 25 mL concentrated HCl:75 mL methanol). HPLC was performed with a μBondapak C_{18} column in a mixture of two solvents: A) 0.005 M tetrabutylammonium hydroxide in water, with pH adjusted to 7.4 with 2% phosphoric acid, and B) 0.005 M tetrabutylammonium hydroxide in methanol, with an equal volume of phosphoric acid as added in solvent A, with a ratio of 90:10 (A:B), at a flow rate of 1 mL/minute. Ultraviolet detection was at 340 nm.

Elemental analyses for carbon, hydrogen, nitrogen, sulfur, and chlorine were in agreement with theoretical values for a mixture containing approximately 80% 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, 14% water, and 6% sodium chloride; the analysis for sodium was slightly low. Weight loss on drying indicated 13.6% ± 0.02% water. TLC indicated one major spot, one minor spot, and one trace impurity. HPLC indicated one major peak and two unresolved impurities. An additional impurity was observed at a detection wavelength of 254 nm. The combined peak area of the impurities varied with wavelength from 1% to 4% relative to the major peak area. HPLC analysis of another lot which was not used in the studies, K021980, obtained from ICN Pharmaceuticals, Incorporated, K&K Labs Division, indicated the presence of three impurities at a detection wavelength of 254 nm. The third impurity of lot K021980 had a peak area of approximately 40% of the major peak area, and was estimated to be present at 16%, based on cumulative analytical data. The impurity was tentatively identified by mass spectrometry as 4,4′-ethylene-2-dianiline sulfonic acid. In lot SW-81605, the third impurity as found by HPLC had the same retention time as the impurity in lot K021980. The overall purity of lot SW-81605 was determined to be approximately 76%.

Stability studies were performed by HPLC with the system described for the purity analysis but with solvent pH adjusted with 1% phosphoric acid and a ratio of 64:36 (A:B), with acetanilide added as an

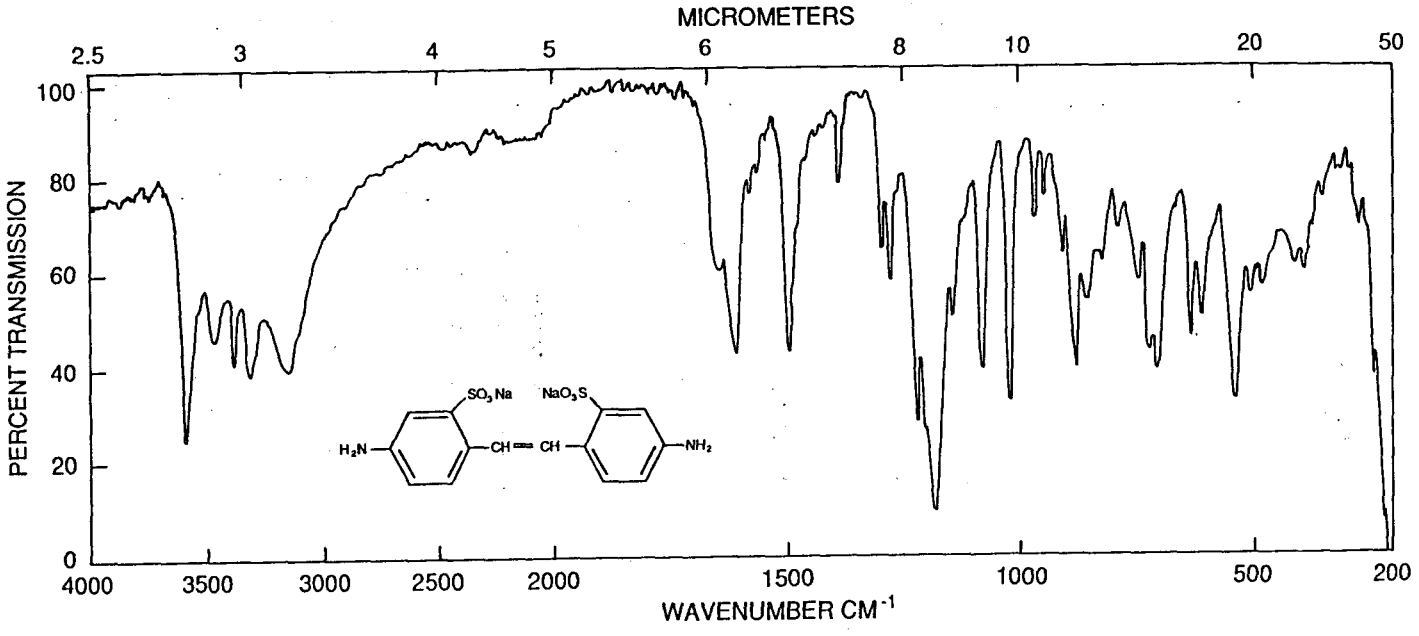
internal standard and ultraviolet detection at 254 nm. The stability studies indicated that 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, when stored protected from light, was stable as a bulk chemical for 2 weeks at temperatures up to 60° C. During the 2-year studies, the stability of the bulk chemical was monitored by the study laboratory using HPLC, with the system above, and with infrared spectrometry. No degradation of the study material was seen throughout the studies.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing appropriate quantities of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, with feed in a Patterson-Kelly twin-shell blender (Table H1). Dose formulations were prepared every two weeks during the 2-year studies.

Homogeneity and stability analyses of the dosed feed preparations were conducted by the analytical chemistry laboratory. For the homogeneity analyses, the formulations were extracted with a solution of methanol in a buffer solution of pH 10.0 ± 0.1, and centrifuged, then further diluted with methanol. The absorbance of the samples was measured versus methanol by ultraviolet spectroscopy at 342 nm. For the stability studies, feed samples were extracted with the same solution used in the homogeneity analyses; the extracts were then mixed with methanol and centrifuged, and were injected into an HPLC system equipped with a μBondapak C_{18} column and a 340 nm detector. The mobile phase was a mixture of two solvents: A) 0.005 M tetrabutylammonium hydroxide in methanol, with pH adjusted to 7.4 with 1% phosphoric acid and B) 0.005 M tetrabutylammonium hydroxide in water, with an equal volume of phosphoric acid added as solvent A, with a ratio of 14:86 A:B, at a flow rate of 1.5 mL/minute. Homogeneity of these formulations was confirmed; stability of the formulation was established for at least 2 weeks when stored in the dark at temperatures up to 5° C. Two 3-week stability studies conducted by the study laboratory using ultraviolet spectroscopy at 342 nm confirmed stability for up to 3 weeks at room temperature.

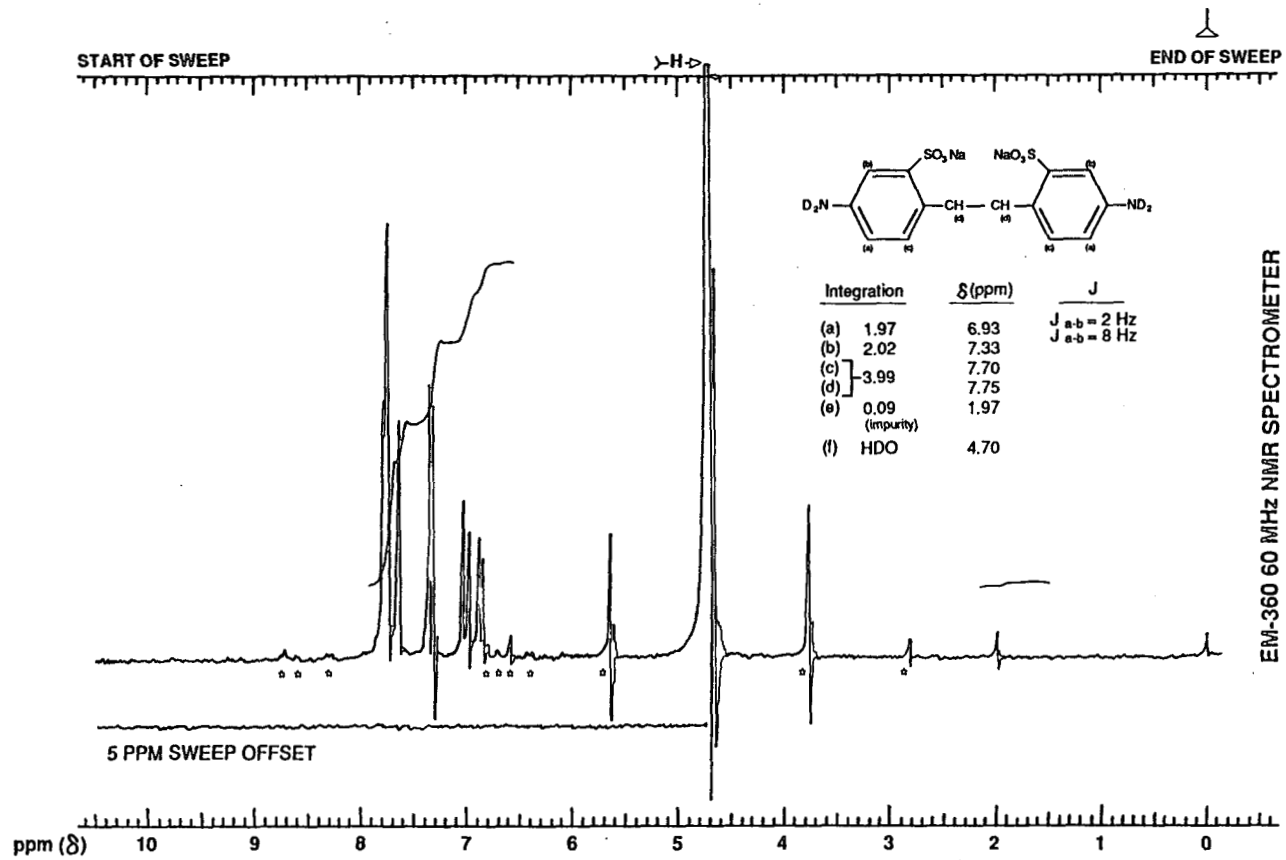
Periodic analyses of the dose formulations of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, were conducted at the study laboratory and at the analytical chemistry laboratory using spectroscopy at 342 nm. Dose formulations were analyzed once during the 14-day studies and twice during the 13-week studies. During the 14-day and 13-week studies, all dose formulations for rats and mice were within the acceptable range of ± 10% of target concentrations (Tables H2 and H3). During the 2-year studies, the dose formulations were analyzed at least once every 8 weeks; 27 of 28 dose formulations for rats and all dose formulations for mice were within the specified 10% of the target concentrations. Results of the dose formulation analyses for the 2-year studies are presented in Table H4. Results of periodic referee analysis performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table H5).



ABSCISSA	ORDINATE		
EXPANSION 1	EXPANSION 1	SCAN TIME 24 min	REP. SCAN – SINGLE BEAM –
SUPPRESSION –	% T 0-100 ABS –	RESPONSE 2	TIME DRIVE – PRE SAMPLE CHOP –
		SLIT PROGRAM 6	OPERATOR T. Witherington DATE 4/3/81

SAMPLE: 4,4′ – Diamino – 2,2′ – stilbenedisulfonic acid, disodium salt Lot No.: SW 81605 Batch No.: 02	REMARKS Trimmer comb in reference beam	SOLVENT –	CELL PATH
		CONCENTRATION 1% (w/w) in a KBr pellet	REFERENCE 046N

FIGURE H1
Infrared Absorption Spectrum of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt



Integration		δ (ppm)	J
(a)	1.97	6.93	$J_{a\text{-}b}$ = 2 Hz
(b)	2.02	7.33	$J_{a\text{-}b}$ = 8 Hz
(c)	3.99	7.70	
(d)		7.75	
(e)	0.09 (impurity)	1.97	
(f)	HDO	4.70	

Spectrum Ampl. 5 x 100
Filter 0.1 sec.
RF Power 0.05 mG

Sweep Time 5 min
Sweep Width 10 ppm or Hz
End of Sweep 0 ppm or Hz

Sample: 4,4' – 2,2' – Diamino– stilbenedisulfonic acid, disodium salt
Lot No.: SW 81605
Batch No.: 02
Solvent: D_2O (TMS)

Remarks: *Sidebands

Operator: J. Davidson
Date: 4/7/81
Spectrum No.: 046N

FIGURE H2
Nuclear Magnetic Resonance Spectrum of 4,4'-Diamino-2,2'-stilbenedisulfonic Acid, Disodium Salt

TABLE H1
Preparation and Storage of Dose Formulations in the Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

14-Day Studies	13-Week Studies	2-Year Studies
Preparation		
A premix with 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, and feed was prepared using a mortar and pestle; premix and remainder of feed were layered into a blender and mixed for 5 minutes with and 10 minutes without an intensifier bar. Dose formulations were prepared weekly.	Same as 14-day studies, but mixed for 20 minutes with an intensifier bar. Dose formulations were prepared every 2 weeks.	Same as 14-day studies, but mixed for 10 minutes without an intensifier bar. Dose formulations were prepared every 2 weeks.
Chemical Lot Number		
SW-81605	Same as 14-day studies	Same as 14-day studies
Maximum Storage Time		
14 days from date of preparation	Same as 14-day studies	Same as 14-day studies
Storage Conditions		
In the dark, refrigerated	Same as 14-day studies	In plastic bags in the dark at 1° C initially; then in plastic bags stored in plastic barrels at approximately 22° C after October 13, 1983
Study Laboratory		
International Research & Development Corporation, Mattawan, MI	Same as 14-day studies	Same as 14-day studies
Referee Laboratory		
Midwest Research Institute, Kansas City, MO	Same as 14-day studies	Same as 14-day studies

TABLE H2
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 14-Day Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	% Difference from Target
Rats				
1 June 1981	5 June 1981	6,250	5,930	−5
		12,500	12,750	+2
		25,000	26,050	+4
		50,000	52,750	+5
		100,000	98,700	−1
	15 June 1981	100,000[b]	97,200	−3
		100,000[c]	101,500	+2
Mice				
5 June 1981	10 June 1981	6,250	6,005	−4
		12,500	12,300	−2
		25,000	25,200	+1
		50,000	52,250	+4
		100,000	99,650	0

[a] Results of duplicate analyses
[b] Samples stored in occupied cage until 10 June 1981
[c] Samples stored in animal room until 10 June 1981

TABLE H3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 13-Week Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Date Prepared	Date Analyzed	Target Concentration[a] (ppm)	Determined Concentration[b] (ppm)	% Difference from Target
15 September 1981	17 September 1981	6,250	5,800	-8
		12,500	11,800	-6
		25,000	24,400	-2
		50,000	49,900	0
		100,000	97,900	-2
27 October 1981	29 October 1981	6,250	6,130	-2
		12,500	12,300	-2
		25,000	25,300	+1
		50,000	51,600	+3
		100,000	104,000	+4

[a] Target concentrations: 6.25 mg/g = 6,250 ppm; 12.5 mg/g = 12,500 ppm; 25.0 mg/g = 25,000 ppm; 50.0 mg/g = 50,000 ppm; 100 mg/g = 100,000 ppm.
[b] Results of duplicate analyses

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Date Prepared	Date Analyzed	Target Concentration[a] (ppm)	Determined Concentration[b] (ppm)	% Difference from Target
Rats				
24 February 1983	25 February 1983	12,500	11,800	−6
		25,000	24,100	−4
21 April 1983	21 April 1983	12,500	11,900	−5
		25,000	23,800	−5
16 June 1983	21 June 1983	12,500	12,000	−4
		25,000	23,000	−9
11 August 1983	18 August 1983	12,500	12,300	−2
		25,000	23,400	−7
29 September 1983	30 September 1983	12,500	12,400	−1
		25,000	23,900	−4
25 November 1983	28 November 1983	12,500	12,000	−4
		25,000	25,800	+3
19 January 1984	24 January 1984	12,500	12,400	−1
		25,000	25,000	0
15 March 1984	15 March 1984	12,500	12,400	0
		25,000	25,000	0
10 May 1984	11 May 1984	12,500	12,800	+2
		25,000	24,700	−1
21 June 1984	26 June 1984	12,500	13,600	+8[c]
		25,000	25,400	+2
16 August 1984	17 August 1984	12,500	12,600	+1
		25,000	25,900	+3
11 October 1984	16 October 1984	12,500	12,000	−4
		25,000	25,000	0
6 December 1984	6 December 1984	12,500	11,100	−13[d]
		25,000	23,600	−6
10 December 1984[e]	11 December 1984	12,500	12,200	−2
31 January 1985	31 January 1985	12,500	12,200	−2
		25,000	24,000	−4

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
Mice				
14 February 1983	15 February 1983	6,250	6,000[f]	−4
		6,250	6,000[g]	−4
		6,250	6,100[h]	−2
		12,500	12,300[f]	−2
		12,500	12,300[g]	−2
		12,500	12,200[h]	−2
28 February 1983	2 March 1983	6,250	6,200	0
11 April 1983	13 April 1983	6,250	6,500	+4
		12,500	11,500	−8
6 June 1983	8 June 1983	6,250	5,900	−6
		12,500	12,300	−2
1 August 1983	2 August 1983	6,250	5,900	−6
		12,500	12,000	−4
26 September 1983	27 September 1983	6,250	5,900	−6
		12,500	12,200	−2
31 October 1983	2 November 1983	6,250	6,400[f]	+2
		6,250	6,200[g]	−2
		6,250	6,300[h]	0
		12,500	12,700[f]	+2
		12,500	12,200[g]	−2
		12,500	12,600[h]	+1
14 November 1983	16 November 1983	6,250	6,000	−3
		12,500	12,200	−2
9 January 1984	11 January 1984	6,250	6,300	+1
		12,500	12,800	+2
5 March 1984	8 March 1984	6,250	6,000	−3
		12,500	12,000	−4
30 April 1984	1 May 1984	6,250	6,300	+1
		12,500	12,500	0
25 June 1984	26 June 1984	6,250	6,200	−1
		12,500	13,000	+4
20 August 1984	21 August 1984	6,250	6,000	−4
		12,500	12,000	−4
15 October 1984	16 October 1984	6,250	6,400	+3
		12,500	12,100	−3
11 December 1984	11 December 1984	6,250	6,500	+4
		12,500	12,800	+2

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt (continued)

[a] Target concentrations for rats: 12.5 mg/g = 12,500 ppm; 25.0 mg/g = 25,000 ppm. Target concentrations for mice: 6.25 mg/g = 6,250 ppm; 12.5 mg/g = 12,500 ppm
[b] Results of duplicate analyses
[c] Mean of two duplicate analyses conducted on 26 and 27 June 1984
[d] Sample remixed
[e] Analysis results of remix
[f] Sample selection from top of twin-shell blender
[g] Sample selection from middle of twin-shell blender
[h] Sample selection from bottom of twin-shell blender

TABLE H5
Results of Referee Analysis of Dose Formulations in the 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory[a]	Referee Laboratory[b]
28 February 1983	6,250	6,200	6,350 ± 350
1 August 1983	12,500	12,000	11,500 ± 100
5 March 1984	12,500	12,000	12,700 ± 200
15 October 1984	6,250	6,400	6,160 ± 70

[a] Results of duplicate analysis
[b] Results of triplicate analysis. Mean ± standard deviation

APPENDIX I
FEED AND COMPOUND CONSUMPTION

TABLE I1 Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 226

TABLE I2 Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 227

TABLE I3 Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 228

TABLE I4 Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 229

TABLE I1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm		12,500 ppm			25,000 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
3	16.5	209	17.1	207	1,031	16.9	205	2,065
6	16.2	280	16.6	275	753	17.8	274	1,625
7	16.4	290	17.0	283	753	14.4	279	1,286
10	16.8	330	16.9	326	648	17.2	321	1,342
11	18.5	345	17.2	336	640	17.5	330	1,329
14	15.6	366	15.5	360	538	15.9	349	1,136
18	15.9	382	16.5	377	546	15.6	364	1,073
22	16.9	381	17.3	379	571	17.0	356	1,195
26	15.0	409	15.7	407	483	16.9	393	1,076
30	16.6	426	16.4	423	487	17.1	403	1,058
34	16.3	434	15.8	428	463	16.5	412	1,000
38	14.9	440	14.8	439	423	15.5	424	912
42	16.5	450	16.9	450	468	17.5	432	1,010
46	16.1	460	16.0	459	435	17.0	441	967
50	16.5	463	16.8	460	457	17.4	447	974
54	16.7	472	17.5	471	465	16.6	458	908
58	16.7	486	17.5	475	460	18.4	459	1,001
62	15.4	478	15.8	475	416	16.1	459	880
66	15.9	479	15.0	473	397	16.1	461	874
70	15.0	486	15.6	471	413	16.0	454	882
74	14.0	485	15.5	468	414	15.6	456	855
78	14.8	473	15.8	466	425	15.6	455	856
82	15.0	473	15.1	464	405	16.0	454	881
86	14.5	478	15.9	464	428	15.9	448	887
90	15.1	469	15.6	468	416	15.9	454	878
92	14.3	455	15.8	461	428	15.7	447	878
94	14.4	458	15.5	457	422	15.9	455	876
96	14.3	455	14.1	457	385	15.7	456	859
100	12.9	439	13.8	440	392	15.8	449	879
102	13.6	429	16.1	441	458	15.2	437	872
104	16.2	434	14.7	435	422	15.6	437	889
Weeks 1-13:								
Mean	16.9	291	17.0	285	765	16.8	282	1,529
SD[c]	0.9		0.2		158	1.4		328
CV[d]	5.6		1.4		20.7	8.3		21.5
Weeks 14-52:								
Mean	16.0	421	16.2	418	487	16.6	402	1,040
SD	0.7		0.7		49	0.7		85
CV	4.4		4.6		10.1	4.4		8.1
Weeks 53-104:								
Mean	14.9	466	15.6	462	422	16.0	453	885
SD	1.1		1.0		23	0.7		34
CV	7.1		6.4		5.5	4.4		3.8

[a] Grams of feed consumed per animal per day
[b] Milligrams of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

TABLE I2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm		12,500 ppm			25,000 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
3	11.4	143	11.3	143	989	11.8	144	2,043
7	11.3	179	11.5	177	816	11.5	173	1,663
11	11.6	200	11.4	199	718	11.2	194	1,439
14	10.0	209	10.6	207	644	10.6	203	1,307
18	10.2	210	10.3	213	605	9.9	208	1,188
22	11.4	219	10.7	217	617	10.7	215	1,242
30	11.1	235	11.1	232	597	10.9	226	1,208
34	10.5	239	10.6	234	565	11.0	230	1,196
38	10.4	247	10.6	245	540	10.8	239	1,136
42	11.3	256	11.6	248	584	11.4	243	1,172
46	11.4	261	11.0	257	537	11.4	251	1,136
50	11.9	269	11.3	262	540	11.3	259	1,088
54	12.8	286	12.6	276	573	12.8	272	1,177
58	12.2	297	12.2	286	534	13.0	280	1,159
62	12.7	306	12.4	294	528	12.7	291	1,094
66	11.9	311	11.5	304	473	12.1	297	1,018
70	10.4	323	11.7	312	470	11.9	304	975
74	11.6	333	12.0	322	466	12.2	314	974
78	11.8	336	11.6	331	439	11.7	326	897
82	11.3	342	11.8	334	442	12.3	326	941
86	11.5	344	12.0	336	447	12.2	328	930
92	11.9	345	12.8	340	472	12.9	331	971
96	11.9	347	12.4	344	450	12.8	336	955
100	11.4	349	12.1	337	451	12.3	334	916
104	11.6	340	11.8	340	433	10.8	337	804
Weeks 1-13:								
Mean	11.4	174	11.4	173	841	11.5	171	1,715
SD[c]	0.2		0.1		137	0.3		305
CV[d]	1.3		0.8		16.3	2.6		17.8
Weeks 14-52:								
Mean	10.9	238	10.9	235	581	10.9	230	1,186
SD	0.7		0.4		38	0.5		64
CV	6.0		3.7		6.6	4.3		5.4
Weeks 53-104:								
Mean	11.8	328	12.1	320	475	12.3	314	985
SD	0.6		0.4		43	0.6		105
CV	5.2		3.3		9.0	4.8		10.6

[a] Grams of feed consumed per animal per day
[b] Milligrams of 4,4′-diamino-2,2′-stilbenedisulfonic acid consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

TABLE I3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm		6,250 ppm			12,500 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
3	3.6	24.1	3.4	24.3	880	3.5	24.2	1,799
7	3.9	28.0	4.0	28.0	883	3.9	27.0	1,810
11	3.8	30.1	3.6	30.2	747	3.8	29.8	1,603
14	3.7	31.5	3.8	30.8	778	4.2	31.4	1,658
18	3.9	32.0	3.8	32.3	735	3.8	32.5	1,452
26	3.7	33.3	3.8	33.0	713	3.7	32.5	1,425
30	3.6	33.7	3.6	33.4	683	3.7	33.3	1,389
34	3.6	34.6	3.3	34.7	589	3.8	34.2	1,372
38	3.6	35.7	3.6	34.9	653	3.8	34.7	1,381
42	4.7	35.7	4.9	35.1	877	4.8	35.2	1,702
46	5.5	35.9	5.3	35.5	925	5.0	35.2	1,784
50	5.1	37.4	5.1	36.3	884	5.3	36.1	1,827
54	5.2	40.1	5.4	38.8	871	5.5	38.0	1,796
58	5.7	37.2	5.5	37.9	913	5.7	37.7	1,880
62	5.0	40.3	5.6	40.3	862	4.9	38.3	1,592
66	4.3	40.3	4.4	39.8	690	4.5	39.5	1,412
70	4.5	41.8	4.5	41.5	678	4.5	41.6	1,352
74	4.1	42.8	4.4	42.1	647	4.5	41.8	1,358
78	3.7	42.6	4.2	42.3	616	4.2	42.4	1,226
86	4.7	41.7	4.5	40.9	690	4.6	40.7	1,413
92	5.3	40.7	5.3	39.8	833	5.8	39.4	1,835
96	4.7	39.9	4.6	39.5	728	4.8	38.8	1,531
100	4.8	39.8	4.8	39.3	760	5.0	38.6	1,622
104	5.5	38.3	5.5	37.8	907	5.6	37.1	1,891
Weeks 1-13:								
Mean	3.8	27.4	3.7	27.5	836	3.7	27.0	1,738
SD[c]	0.2		0.3		78	0.2		116
CV[d]	4.6		7.4		9.3	6.0		6.7
Weeks 14-52:								
Mean	4.2	34.4	4.1	34.0	760	4.2	33.9	1,554
SD	0.7		0.7		115	0.6		186
CV	17.7		18.0		15.2	14.9		12.0
Weeks 53-104:								
Mean	4.8	40.5	4.9	40.0	766	5.0	39.5	1576
SD	0.6		0.5		106	0.5		231
CV	12.4		10.9		13.8	11.1		14.6

[a] Grams of feed consumed per animal per day
[b] Milligrams of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

TABLE I4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	0 ppm		6,250 ppm			12,500 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
3	3.2	19.0	3.2	18.7	1,080	3.4	18.9	2,276
7	3.4	21.6	3.5	21.0	1,045	3.7	21.3	2,160
11	3.2	23.1	3.2	23.3	867	3.3	23.1	1,808
14	3.5	24.3	3.4	24.1	882	3.4	24.3	1,774
18	3.2	25.6	3.3	26.3	794	3.5	25.1	1,766
22	3.0	26.7	3.1	26.7	715	3.4	25.4	1,661
26	3.2	27.2	3.3	27.3	745	3.3	25.9	1,572
30	3.3	27.5	3.5	27.6	794	3.4	26.3	1,629
34	3.0	29.3	3.2	29.9	673	3.5	28.1	1,537
38	3.3	30.9	3.2	31.4	635	3.4	29.0	1,486
42	2.9	31.5	3.2	32.0	635	3.3	30.1	1,376
46	3.6	29.8	3.6	31.7	710	3.7	30.4	1,508
50	3.4	32.1	3.5	32.6	667	3.5	31.2	1,401
54	3.3	34.5	3.4	34.9	615	3.6	32.6	1,371
58	3.3	33.9	3.4	34.2	618	3.4	32.8	1,286
62	3.4	35.4	3.8	34.7	690	3.7	32.7	1,412
66	3.5	35.4	3.6	34.9	639	3.5	33.2	1,315
70	3.5	37.3	3.4	37.7	571	3.4	34.4	1,250
74	3.3	37.8	3.5	37.6	582	3.4	35.7	1,195
78	2.9	37.7	2.8	38.0	460	3.3	35.5	1,162
82	3.9	37.3	3.4	38.8	548	3.4	36.0	1,163
86	3.6	37.5	3.5	38.3	567	3.5	35.9	1,221
92	4.1	38.0	4.1	38.1	666	4.6	36.1	1,607
96	3.6	37.9	3.6	38.1	583	3.6	35.7	1,272
100	3.8	37.1	3.9	38.0	641	4.0	36.0	1,382
104	4.1	37.5	4.2	37.3	711	4.2	35.6	1,492
Weeks 1-13:								
Mean	3.2	21.2	3.3	21.0	997	3.5	21.1	2,081
SD[c]	0.1		0.2		114	0.2		244
CV[d]	4.0		4.9		11.5	5.0		11.7
Weeks 14-52:								
Mean	3.2	28.5	3.3	29.0	725	3.4	27.6	1,571
SD	0.2		0.2		80	0.1		138
CV	7.3		5.0		11.0	3.4		8.8
Weeks 53-104:								
Mean	3.6	36.7	3.6	37.0	607	3.7	34.8	1,317
SD	0.3		0.4		66	0.4		132
CV	9.4		10.1		10.9	10.8		10.0

[a] Grams of feed consumed per animal per day
[b] Milligrams of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disulfonic salt, consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

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

TABLE J1 Ingredients of NIH-07 Rat and Mouse Ration 232
TABLE J2 Vitamins and Minerals in NIH-07 Rat and Mouse Ration 232
TABLE J3 Nutrient Composition of NIH-07 Rat and Mouse Ration 233
TABLE J4 Contaminant Levels in NIH-07 Rat and Mouse Ration 234

TABLE J1
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 J2
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 J3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.43 ± 0.94	21.0–24.5	25
Crude fat (% by weight)	5.28 ± 0.66	4.2–6.4	25
Crude fiber (% by weight)	3.59 ± 0.32	2.9–4.5	25
Ash (% by weight)	6.65 ± 0.28	6.0–7.3	25
Amino Acids (% of total diet)			
Arginine	1.308 ± 0.606	1.210–1.390	8
Cystine	0.306 ± 0.084	0.181–0.400	8
Glycine	1.150 ± 0.047	1.060–1.210	8
Histidine	0.576 ± 0.024	0.531–0.607	8
Isoleucine	0.917 ± 0.029	0.881–0.944	8
Leucine	1.946 ± 0.055	1.850–2.040	8
Lysine	1.270 ± 0.058	1.200–1.370	8
Methionine	0.448 ± 0.128	0.306–0.699	8
Phenylalanine	0.987 ± 0.140	0.665–1.110	8
Threonine	0.877 ± 0.042	0.824–0.940	8
Tryptophan	0.236 ± 0.176	0.107–0.671	8
Tyrosine	0.676 ± 0.105	0.564–0.794	8
Valine	1.103 ± 0.040	1.050–1.170	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.393 ± 0.258	1.830–2.570	7
Linolenic	0.280 ± 0.040	0.210–0.320	7
Vitamins			
Vitamin A (IU/kg)	11,488 ± 4,665	4,200–22,000	25
Vitamin D (IU/kg)	4,450 ± 1,382	3,000–6,300	4
α–Tocopherol (ppm)	37.95 ± 9.41	22.50–48.90	8
Thiamine (ppm)	20.12 ± 5.09	12.0–37.0	25
Riboflavin (ppm)	7.92 ± 0.87	6.10–9.00	8
Niacin (ppm)	103.38 ± 26.59	65.0–150.0	8
Pantothenic acid (ppm)	29.54 ± 3.60	23.0–34.0	8
Pyridoxine (ppm)	9.55 ± 3.48	5.60–14.0	8
Folic acid (ppm)	2.25 ± 0.73	1.80–3.70	8
Biotin (ppm)	0.254 ± 0.042	0.19–0.32	8
Vitamin B_{12} (ppb)	38.45 ± 22.01	10.6–65.0	8
Choline (ppm)	3,089 ± 329	2,400–3,430	8
Minerals			
Calcium (%)[a]	1.21 ± 0.15	0.87–1.43	24
Phosphorus (%)	0.95 ± 0.06	0.84–1.10	25
Potassium (%)	0.883 ± 0.078	0.772–0.971	6
Chloride (%)	0.526 ± 0.092	0.380–0.635	8
Sodium (%)	0.313 ± 0.390	0.258–0.371	8
Magnesium (%)	0.168 ± 0.010	0.151–0.181	8
Sulfur (%)	0.280 ± 0.064	0.208–0.420	8
Iron (ppm)	361 ± 100	255.0–523.0	8
Manganese (ppm)	91.97 ± 6.01	81.70–99.40	8
Zinc (ppm)	54.72 ± 5.67	46.10–64.50	8
Copper (ppm)	11.06 ± 2.50	8.09–15.39	8
Iodine (ppm)	3.37 ± 0.92	1.52–4.13	6
Chromium (ppm)	1.79 ± 0.36	1.04–2.09	8
Cobalt (ppm)	0.68 ± 0.14	0.490–0.780	4

[a] No measurement was taken for calcium in the lot milled 14 August 1984

TABLE J4
Contaminant Levels in NIH-07 Rat and Mouse Ration

Contaminants	Mean ± Standard Deviation[a]	Range	Number of Samples
Arsenic (ppm)	0.55 ± 0.17	0.18–0.78	25
Cadmium (ppm)[b]	0.12 ± 0.04	<0.10–0.20	25
Lead (ppm)	0.54 ± 0.21	0.24–1.00	25
Mercury (ppm)	<0.05		25
Selenium (ppm)	0.32 ± 0.06	0.21–0.46	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)	9.86 ± 4.84	2.50–22.0	25
Nitrite nitrogen (ppm)	0.89 ± 1.40	<0.10–6.10	25
BHA (ppm)[c]	<2.00		25
BHT (ppm)[c]	2.48 ± 1.26	<1.00–5.00	25
Aerobic plate count (CFU/g)[d]	145,468 ± 148,232	6,600–420,000	25
Coliform (MPN/g)[e]	367 ± 683	<3.00–2,400	25
E. coli (MPN/g)[f]	8.96 ± 29.39	<3.00–150	25
E. coli (MPN/g)[g]	3.08 ± 0.28	<3.00–4.0	24
Total nitrosoamines (ppb)[h]	5.67 ± 5.74	0.80–30.30	25
N-Nitrodimethylamine (ppb)[h]	4.98 ± 5.77	0.50–30.00	25
N-Nitrosopyrrolidine (ppb)[h]	0.69 ± 0.71	0.30–2.70	25
Pesticides (ppm)			
α-BHC[i]	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05	0.06 (26 July 1983)	25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.1		25
Estimated PCBs	<0.2		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.1		25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion[j]	0.15 ± 0.18	0.05–0.81	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

TABLE J4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Four lots (milled on 22 February 1984, 14 March 1984, 9 May 1984, and 13 June 1984) contained 0.20 ppm.
[c] Sources of contamination: soy oil and fish meal.
[d] CFU = colony-forming unit
[e] MPN = most probable number.
[f] Mean, SD, and range exclude one large value of 150 MPN/g obtained in the lot milled on 17 October 1984.
[g] Mean, SD, and range include the value of the lot milled on 17 October 1984.
[h] All values were corrected for percent recovery.
[i] BHC is hexachlorocyclohexane or benzene hexachloride.
[j] Fourteen lots contained more than 0.05 ppm.

APPENDIX K
SENTINEL ANIMAL PROGRAM

METHODS 238
TABLE K1 Murine Virus Antibody Determinations for Rats and Mice in the 13-Week and 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt 240

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 on sera from (sentinel) animals in the study rooms. These animals and the study animals are 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.

Rats

During the 13-week studies, samples for viral screening were collected from five diet control animals of each sex. At the termination of the 13-week studies, the animals were bled. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Incorporated (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM (pneumonia virus of mice)	Study termination
Sendai	Study termination
KRV (Kilham rat virus)	Study termination
H-1 (Toolan's H-1 virus)	Study termination
Complement Fixation	
RCV (rat corona virus)	Study termination
SDA (sialodacryoadenitis virus)	Study termination

During the 2-year studies, 15 F344/N rats of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals from each designated sentinel group were killed at 6, 12, and 18 months. Samples for viral screening at 24 months were collected from five diet control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Incorporated (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM	6, 12, and 18 months
Sendai	6, 12, and 18 months
KRV	6, 12, 18, and 24 months
H-1	6, 12, 18, and 24 months
ELISA	
RCV/SDA	6, 12, 18, and 24 months
Mycoplasma pulmonis	24 months
Mycoplasma arthritidis	24 months
PVM	24 months
Sendai	24 months

Test results are presented in Table K1.

Mice

During the 2-year studies, 15 $B6C3F_1$ mice of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6 and 12 months; three males and four females were killed at 18 months, due to early deaths. Samples for viral screening at 24 months were collected from five diet control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM	6, 12, and 18 months
Reovirus 3	6, 12, and 18 months
GDVII (mouse encephalomyelitis virus)	6 and 12 months
Polyoma virus	6, 12, 18, and 24 months
Sendai	6, 12, and 18 months
MVM (minute virus of mice)	6, 12, 18, and 24 months
Ectromelia virus (mouse pox)	6, 12, and 18 months
K (papovavirus)	24 months
Complement Fixation	
Mouse adenoma virus	6, 12, and 18 months
LCM (lymphocytic choriomeningitis virus)	6, 12, 18, and 24 months
ELISA	
PVM	24 months
Reovirus 3	24 months
GDVII	18 and 24 months
Sendai	24 months
Ectromelia virus	24 months
Mouse adenoma virus	24 months
Mycoplasma pulmonis	24 months
Mycoplasma arthritidis	24 months
MHV (mouse hepatitis virus)	6, 12, 18, and 24 months
Immunofluorescent Antibody	
EDIM (epizootic diarrhea of infant mice)	24 months
MHV	24 months

Test results are presented in Table K1.

TABLE K1
Murine Virus Antibody Determinations for Rats and Mice in the 13-Week and 2-Year Feed Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

	Interval	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
13-Week Studies			
Rats	13 weeks	0/10	None positive
2-Year Studies			
Rats	6 months	0/10	None positive
	12 months	0/10	None positive
	18 months	0/10	None positive
	24 months	2/10	KRV
Mice	6 months	2/10	MHV
	12 months	10/10	MHV
	18 months	6/7	MHV
	24 months	2/10	MHV
		3/10	*M. arthritidis*[a]
		8/10	EDIM

[a] Possible *Mycoplasma arthritidis*

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

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338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Rotenone
346	Chloroethane
347	D-Limonene
348	*a*-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3'-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
385	Methyl Bromide
386	Tetranitromethane
387	Amphetamine Sulfate
388	Ethylene Thiourea
389	Sodium Azide
390	3,3'-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
392	Chlorinated Water and Chloraminated Water
393	Sodium Fluoride
395	Probenecid
396	Monochloroacetic Acid
399	Titanocene Dichloride
401	2,4-Diaminophenol Dihydrochloride
403	Resorcinol
405	C.I. Acid Red 114
406	γ-Butyrolactone
407	C.I. Pigment Red 3
410	Naphthalene
415	Polysorbate 80
419	HC Yellow 4

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